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CORRELATION OF THE LIFE CYCLE OF A PARASITE WITH THE METAMORPHOSIS OF ITS HOST

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Apparently but few instances have been noted where a parasite changes in form *pari passu* with the changes in its host during metamorphosis. It would be interesting to know if such a correlation is a common one. Certainly further knowledge of such adaptations would assist in the identification of some parasites and in a better understanding of their life histories. The object of this paper is to describe what is apparently a clear instance of such a correlation between a gregarine parasite and its host.

THE HOST AND METHODS OF HANDLING IT

The host of the gregarine under discussion is the fly, *Sciara coprophila,* a member of the family Mycetophilidae. It commonly occurs on house plants, feeding on their roots and breeding in the loose upper layers of the dirt in which the plants are grown. It may also be found under leaves and in the moist earth at the base of trees. The height of the breeding season is in April and early May, although it begins in March and continues at a slow rate all summer. The larva is about 6 mm. long at the maximum size, with a body color of cream or white and a black head. The pupa is half the length of the larva, and varies from deep cream to brown according to age. The adult female fly averages 3 mm. in length and the male, 2.5 mm. The duration of the larval existence is from eleven to twelve days; of the pupal, six days. The eggs hatch in about a week. All these figures fluctuate slightly with different conditions of food and temperature. The host has a complete metamorphosis, a matter which is of special significance in that it has an important bearing on the development of the parasite. In each of the three phases of metamorphosis there is accommodation for only a definite and limited phase of the parasite's cycle. For example, the trophozoite, or growing gregarine, is confined to the larva; the conjugating gregarine and the preliminary cyst formation, to the pupa; and the spore forming and sporozoite development, to the adult fly. The growing time of the parasite coincides with the feeding and growing time of the host; the preparation for reproduction

falls simultaneously with the period of sexual differentiation in the host; and the production of spores, the last step in the reproduction of the parasite, takes place in the adult fly which is itself ripe for the fertilization program.

So far as the writer knows such a correlation has not been thus far described for gregarines nor for any other parasite. Gregarines usually complete their entire cycle in the larval stage of the host, in case it is a host with complete metamorphosis or if they fail to form spores at the larval stage they may do so in the pupa or even in the adult. Léger (1892) found that certain gregarines hibernate with the host and continue development only when the host resumes activity. But in all these cases the element of chance, the chance of age operates; there is no regularity, no timed correlation of homologous phases of two animals as we have in the present instance.

All stages of the host were observed and with very few exceptions they showed heavy infections. Three lots have been used in this paper: Lot 1, collected by Mr. H. B. Hungerford on house plants, April 19, 1918; Lot 2, collected by the writer on a greenhouse snapdragon, May 16, 1920; Lot 3, collected by the writer on the soft earth at the base of trees growing on the campus of the University of Kansas, June, 1920. Several other species of Sciara, as well as some of the more distantly related members of the family Mycetophilidae, have been examined for gregarine infection, but none has been found thus far.

The infected larvae, pupae and flies were examined in some cases in smears immediately after capture. The freshly collected host was placed in a drop of normal salt solution. With a fine lancet the body was cut behind the jaws and with a needle the digestive tract and its accompanying glands were pulled out upon the slide. In the larvae the larger gregarines are visible through the intestinal walls and through the transparent cells of the ceca; and in the adult fly the dark spherical cysts show distinctly through the wall of the otherwise empty stomach. Infection, except at its inception, is therefore, easy to determine.

Other material was fixed before examination. Hot Schaudinn fluid was found best for preserving guts to be mounted whole, and both borax carmine and Mallory's chloride of iron hematoxylin were used for staining these mounts. Hot Bouin's fluid was employed for material to be sectioned. This material, which consisted of entire larvae, pupae and flies, was immersed two hours in the fixative, the chitinous body wall being first pierced with a needle in several places. Sections were cut 7 to 10μ thick and were stained by the Heidenhain iron-hematoxylin method as well as by Mallory's chloride of iron hematoxylin. Both gave satisfaction, but Mallory's stain was more often used because the process can be completed in half an hour and is superior for spore staining.

THE GREGARINE OF THE LARVAL HOST

When an infected larval intestine is drawn from the body upon a slide in normal salt solution, almost invariably large gregarines will be seen pushing through the tissue to free themselves. If the intestinal wall is broken by pressing the cover glass still other gregarines appear. These vary in size from very small to very large forms, but a glance will show them all to be in the trophozoite or growing stage.

By examining stained sections of the host of the same age the normal location of the parasites can be readily determined. The very young are in the cells which line the intestinal tract and the ceca (Fig. 1); and the older forms, the so-called sporonts, are free in the lumen of these organs. Thus we see that the parasite begins its development intracellularly. The time between the ingestion of the sporozoite and its entrance to the cell must be very short, because few free sporozoites are found in the intestine, while the very young intracellular trophozoites are numerous in the larvae. At its earliest intracellular stage (Fig. 2) the gregarine is not much larger than the nucleus of the cell which it has invaded, but by rapid growth it soon fills the cell and crowds the nucleus to one side (Figs. 3, 5, 6, 7). Intracellular development takes place impartially in the ceca and in the midgut. Usually both places are invaded. Likewise the sporonts occupy the lumen of both of these organs but wander into the intestine during the metamorphosis of the larva into the pupa.

From a smooth and very minute sphere measuring 10μ in diameter the parasite grows to fifteen or twenty times that diameter before bursting the cell membrane. Meanwhile it is developing a polycystid structure, which may be easily overlooked, because of the folded condition imposed by the narrow cell limits (Figs. 5, 6, 7, 8, 35). The epimerite is small and button-like and it is doubtful whether it is ever functional because it disappears before normal emergence of the parasite from the cell. Another structure is also lost to the gregarine about this time, the septum between the protomerite and the deutomerite. Although vestigial in character both the epimerite and the protomerite are a definite part of the early development of this gregarine and show its phylogenetic relationship to the polycystids.

By the time the sporont is free in the lumen it retains only a knob-like remnant of a protomerite and the epimerite has vanished, sometimes leaving a starlike scar to mark its point of attachment. The deutomerite of the full grown sporont is relatively long, narrow and spatulate, tapering sharply posteriorly. In it, about midway, lies the nucleus containing from one to three or more chromatin bodies. Occasionally, deeply stained granules are scattered through the cytoplasm. Sporonts often attain a length of 200μ in the intestinal cavity. Such forms usually lie close against the wall but are not attached to

it (Fig. 34). The head end is toward the head of the host unless there are large numbers of parasites, in which case the gregarines swing the head end toward the intestinal wall. The sporonts are always solitary.

That many separate infections have taken place is indicated by the wide variation in the development of the parasites. Some are in the youngest intracellular stage while others are apparently mature sporonts; but, not withstanding this, no time is gained in the larval host. All sporonts have met a closed door and must postpone further development until the host passes over into the pupal phase. No larva, at any age, was found to harbor parasites later than the sporont stage.

THE GREGARINE CF THE PUPA

There seems to be a period of quiescence in the gregarine's development at the end of the larval phase, for we find the intestine of the late larva crowded with sporonts of various size and very few intracellular forms. This pause is preparatory to the next phase, pseudo-conjugation, which in most gregarines proceeds in the larva. But in the present case the process of development stops short in the larva and recommences apparently under the stimulus of the pupal stage of the host.

A change of position comes with the pupal phase. The sporonts which have lain with their anterior end toward the anterior end of the host begin to writhe about with a snakelike movement. This motion is visible through the gut wall and after some time accomplishes the union of the sporonts in pairs. Previous to this joining so great an elongation of the sporonts has taken place that they are hardly recognizable as the same animal. They have also become narrow except at the anterior end, which has widened perceptibly. The union is head to head with usually no dimorphism of conjugants. Occasionally one sees a ball and socket union, such as described by Duke (1910), and sometimes a pair consists of one short and one long conjugant, but these variations are not constant enough to be of any significance. Figs. 12 to 17 show the various stages of pseudo-conjugation which occur in the pupa.

A shortening of the copula next follows, resulting in a bilobed sphere. There seems to be a condensation of the entire mass at this time, though it proceeds most rapidly at the anterior ends bringing the nuclei of the two animals close together. The posterior tip is the last to lose itself in the rounded mass and remains for a time protruding like a tail. The pupa of the mid phase shows a varied picture (Fig. 14). The copulae are in every stage from late contraction to early segmentation of the bispherical cysts.

Sometimes before the cyst is yet rounded the nucleus of each half has begun to divide. This continues until the cytoplasm is well dotted

with chromatin patches, and is itself condensing around them to form the so-called pearl stage of the gregarine life history. Unfortunately, material was not available for a detailed study of the processes immediately following this, and the union of the gametes is therefore undetermined. All divisions are plainly mitotic (Fig. 21).

The sphere early begins a revolving movement which is connected with the formation of the cyst wall. This wall makes its appearance first as a granular secretion around the sphere (Fig. 20). There is some doubt about its composition. In some of the sections it has a distinctly cellular look after it is well along in development, but the initial structureless secretion together with the reports of such authors as Léger and Duboscque (1909) and Siedlecki (1900) makes the gelatinous theory of structure more probable. In the older cysts the wall is stratified, deeply pigmented and opaque.

THE GREGARINE OF THE FLY

Perhaps the most noteworthy changes that the parasite undergoes in the fly is the formation of the spores. These have begun at the time of the pearl stage in the pupa, but little more has been accomplished there than the division of the sphere into minute bodies. It is possible that these bodies are gametes and unite for the production of the spore, as with *Monocystis agilis* and many others. But conjugation was not observed and I am inclined to believe that the spores are not zygotes. The spore wall appears first irregular in outline and very thin, but as the spores move further apart the wall thickens, assumes a bi-conical form and at last develops a winged edge along one side (Fig. 31). Division of the nucleus begins about this time and continues until in the oldest flies the spores contain frequently eight patches of chromatin.

None of these changes can be seen in the early fly except in sections because the spores are all contained in cysts. These cysts are often so numerous in the stomach of the host that they distort that organ (Figs. 23, 24). But later the cyst walls disintegrate and the spores are loosened. There are no ducts for the passage of these spores and there is no definite break; the wall simply crumbles away from the contents. For a time the boat-shaped bodies within hang together but at last they become so widely distributed that in an old fly there is no region of the digestive tract from esophagus to rectum which does not harbor the loose spores (Figs. 25 and 26).

No further development of the spore was observed in the host, but when cysts are placed in normal salt solution the sporozoites become free within a few hours. The spores were not isolated and the exact number of sporozoites to the spore was not determined. Our assumption of eight rests upon the eight divisions of the spore chromatin as

previously described. Infection of the new host is probably through the ingestion of spores and not of cysts, since the cysts have broken down before the natural death of the host.

CLASSIFICATION AND AFFINITIES

The genus Schneideria, in which I have placed the gregarine of this paper, was created by Léger in 1892 and contains up to the present only three species: *S. mucronata, S. coronata* and *S. praecox*. I have found no description of *S. praecox*.

The characteristics of the genus as Léger gives them are:

1. Epimerite in the form of a thick, horizontal dish with milled border.
2. Sporonts, solitary.
3. Sporonts with a single segment; i. e., no protomerite in the adult stage.

The present gregarine possesses the second and third characteristics, but the epimerite is too rudimentary to be described as a thick milled disk. It is merely a button-like elevation of but short duration. This difference may justify the creation of a new genus, but if so, I shall leave it to the systematists.

That the specimen is neither *S. mucronata* nor *S. coronata* I have shown by the following table. It is without doubt a new species which I have called *S. metamorphosa* because of the close correlation of its life cycle with the metamorphosis of its host.

	S. mucronata	*S. coronata*	*S. metamorphosa*
Host	*Bibio marci*	*Sciara nitidicollis*	*Sciara coprophila*
Development	May complete entire developed cycle in larva	May complete entire developed cycle in larva	Requires all three phases: larva, pupa and imago for completion of cycle
Location in host	Limited to cells of ceca until time for encystment, only then passing into intestine	Limited to cells of ceca until time for encystment, only then passing into intestine	Not limited to ceca for early developed phases, but using both ceca and intestine
Epimerite	Epimerite with milled border and a short style at center, functions for a short time after sporont emerges from cell	Epimerite more sessile than in *S. mucronata*, a milled border and possessing no style, functions for a short time after sporont emerges from cell	Epimerite a mere bud, plain border and no style, disappears while gregarine is still in cell, never functions at any time
Form of Cyst	Bi-spherical	Unknown	Spherical
Maximum size	800 μ	1,000 μ	300 μ

Léger found *S. mucronata* and *S. coronata* to be true dicystids during their intracellular existence and for a short time after emergence from the cell. The epimerite is functional for a while before it is shed, leaving the sporont a monocystid in form. This cycle he found slightly modified in the genera Gametocystis and Sphaerocystis. In these the

epimerite is more ephemeral than in the above cases, disappearing completely by the time the intracellular growth is attained. There is no trace of the dicystid nature at the time of emergence.

Schneideria metamorphosa conforms to both Gametocystis and Sphaerocystis as regards the ephemeral epimerite, but during the intracellular phase it possesses besides this an equally distinct and ephemeral protomerite. For a brief time the gregarine is a true polycystid. This is more evidence for Léger's statement that "The true dicystids are united to the polycystids by insensible gradations."

Each of the three phases of the host's development is linked with a definite and limited phase of the parasite's development. And a still closer view shows this correlation to be physiologic as well as morphologic: the parasite and host feed and grow at the same time; have their quiescent periods at the same time; and carry on the propagation program simultaneously. The writer suspects that this extraordinary adaptation of the parasite's life cycle to fit that of its host may be frequent in the gregarine group and may explain the incompleteness of so many gregarine life histories.

SUMMARY AND CONCLUSIONS

In the fly, *Sciara coprophila,* a new host has been found for a gregarine previously noted only in *S. nitidicollis.*

It is the fourth species recorded in the rare genus Schneideria Léger, and the only one found outside of France.

It differs from the other recorded species in that (*a*) its intracellular phase is polycystid; (*b*) the epimerite is never functional; and (*c*) both epimerite and protomerite are shed before gregarine leaves the cell.

Its life cycle presents a remarkable correlation with that of its host; it begins as an intracellular parasite in the midgut of the larva of the fly, *Sciara coprophila.* Here it undergoes a polycystid development, possessing an epimerite, a protomerite and a deutomerite. While still inside the cell all divisions atrophy except the deutomerite, and the parasite emerges a monocystid. In the larva the gregarine goes no farther than the sporont stage. In the pupa, sporonts, solitary up to this time, unite in twos, head to head, shorten into spheres and begin segmentation, meanwhile laying down a cyst wall. In the adult fly the formation of the spores is completed and the spore chromatin divides preliminary to the production of the sporozoites.

Acknowledgements are due to Dr. H. B. Hungerford, Department of Entomology, University of Kansas, for a large supply of material and for important observations on the living gregarines; and to Dr. H. B. Ward, Department of Zoology, University of Illinois, and Dr. Minnie Watson Kamm, for the loan of literature.

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Explanation of Plates

All drawings were made with camera lucida except Fig. 23, which is a free hand drawing.

Explanation of Plate XVIII

All figures from larva of *Sciara coprophila*

Fig. 1.—Median longitudinal section of larva of *Sciara coprophila* showing gregarines in all states of growth. Some occupy the cells of the intestinal and cecal walls and others are free in the lumen of the intestine.

Figs. 2, 3 and 4.—Sporozoite just before and after entering the cells of the intestine.

Figs. 5, 6 and 7.—Early intracellular stages of the parasite undergoing the polycystid phase. The protomerite and deutomerite are at first nearly equal in size. Note that the epimerite is functionless.

Fig. 8.—A late intracellular phase. The deutomerite is gaining length over the protomerite.

Fig. 9.—Small gregarine from the cecal lumen; very unusual remnant of epimerite which is ordinarily shed or absorbed inside the host cell.

Fig. 10.—Enlarged section of mid gut showing various stages of development of the parasite.

Fig. 11.—One of the large free sporonts measuring over 200 μ long. Note slight constriction at anterior end and lack of septum. On the anterior end is the scar of the lost epimerite.

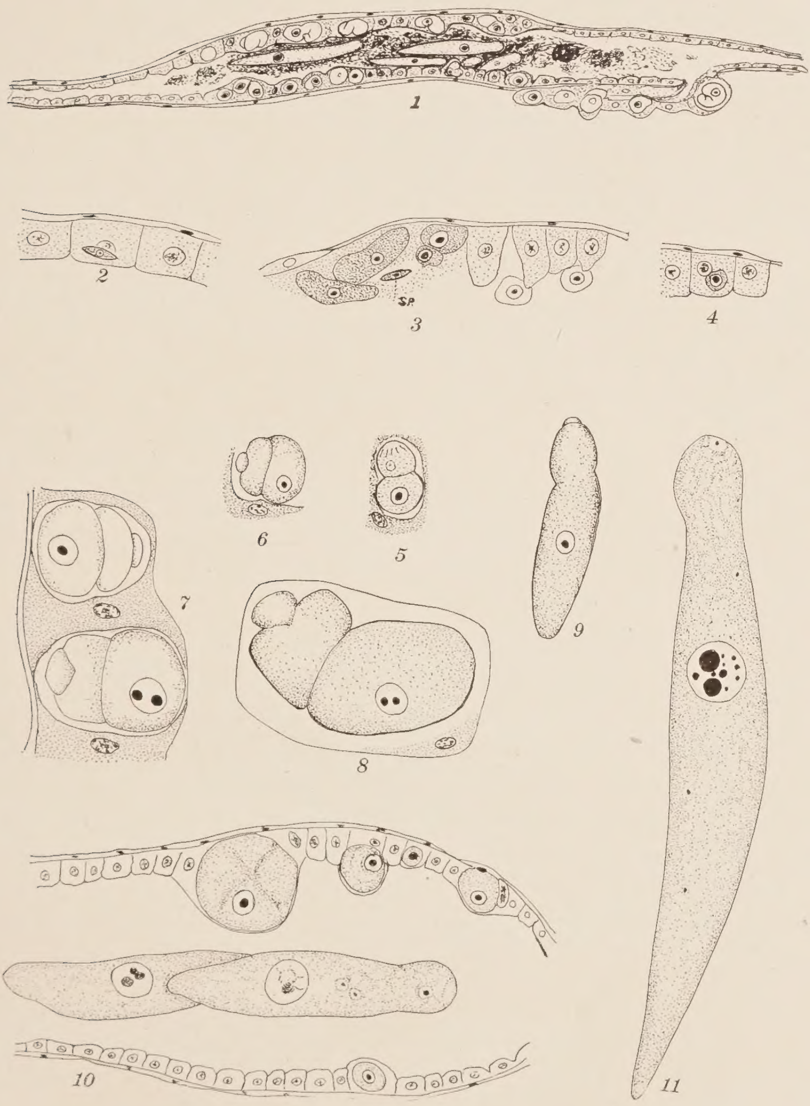

PLATE XVIII

Explanation of Plate XIX

All figures from pupa of *Sciara coprophila.*

Fig. 12.—Free hand sketch of stomach of late pupa showing gregarines in pseudo-conjugation bursting through wall after accidental puncture.

Fig. 13.—Median longitudinal section of early pupal mid gut showing copulae just getting together.

Fig. 14.—Stage later than Figure 13; contraction of copulae has begun; some are in segmentation stage.

Fig. 15.—Pair of conjugants extended.

Fig. 16.—Beginning of contraction of conjugants.

Fig. 17.—Continuation of contraction of conjugants.

Figs. 18 and 19.—The early sphere.

Fig. 20.—Early segmentation of sphere, and beginning of cyst wall.

Fig. 21.—Drawn under oil immersion to show mitosis; cyst wall in process of formation.

Fig. 22.—Enlarged view to show method of union of two conjugants.

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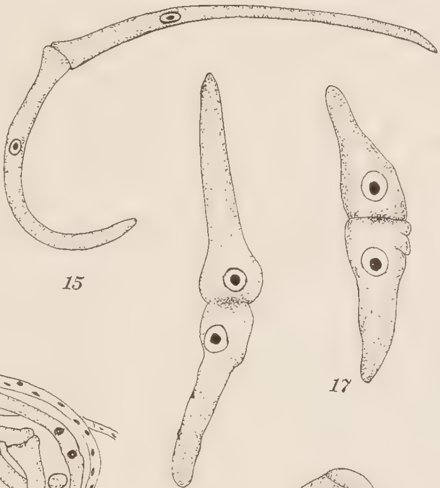

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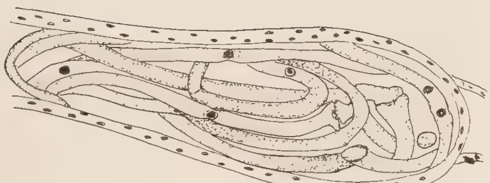

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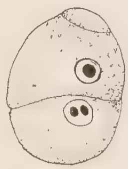

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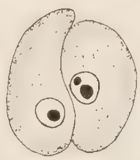

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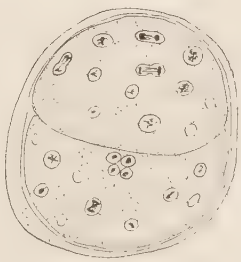

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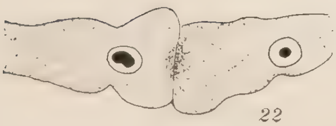

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PLATE XIX

EXPLANATION OF PLATE XX

All drawings from the adult fly.

Fig. 23.—Free hand drawing of fly stomach showing cysts through body wall.

Fig. 24.—Section of fly stomach showing cysts and otherwise empty lumen.

Fig. 25.—Stomach and intestine of fly showing breaking up of cysts and dissemination of spores even to rectum.

Fig. 26.—Fly stomach showing cysts completely disintegrated with spores widely scattered. Even esophagus of this specimen showed loose spores. Absence of food in the digestive tract shows that these spores date from larval infection.

Fig. 27.—Early cyst showing irregular spores.

Fig. 28.—Spores loosening from each other and cyst wall breaking away.

Fig. 29.—Boat-shaped spores fully formed and loose inside inner cyst wall.

Fig. 30.—An enlarged view of the uninucleate spores.

Fig. 31.—Division of nucleus preliminary to sporozoite formation.

Fig. 32.—A free spore.

PLATE XX

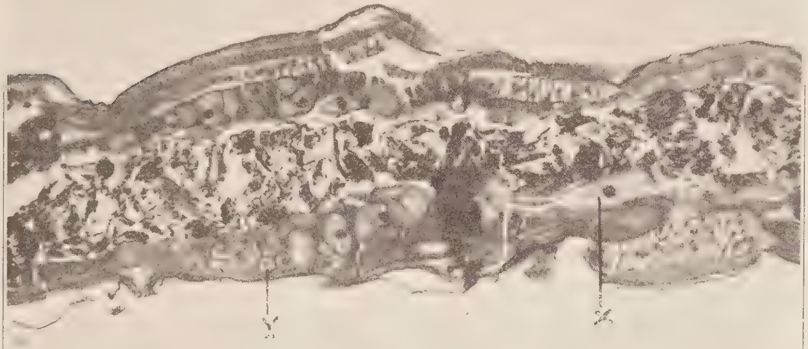
Explanation of Plate XXI

Photomicrographs of median longitudinal sections of larva.

Fig. 33.—Intracellular development of gregarine in intestinal wall of larval host. At x a sporont is lying free in the lumen. $\times$ 160.

Fig. 34.—Typical adult sporont with tapering posterior and knob-like anterior end, lying in intestinal lumen. $\times$ 160.

Fig. 35.—Highly magnified trophozoite (y) still in intestinal cell. It shows the polycystid form through which *Schneideria metamorphosa* passes in its intracellular growth stage. The protomerite is distinct. The epimerite is the button which fits wedge-like into its free end, and is somewhat more difficult to distinguish. $\times$ 350.



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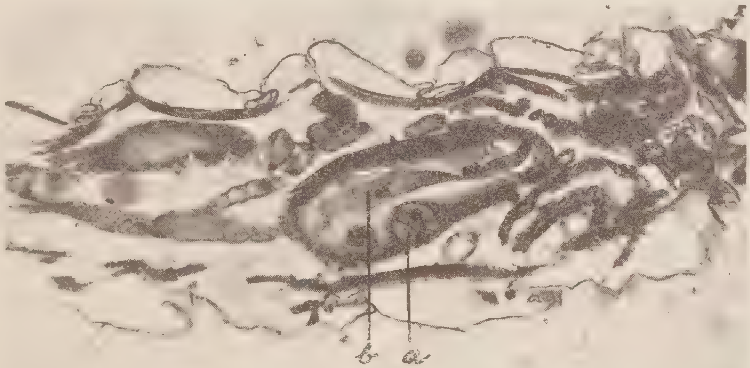

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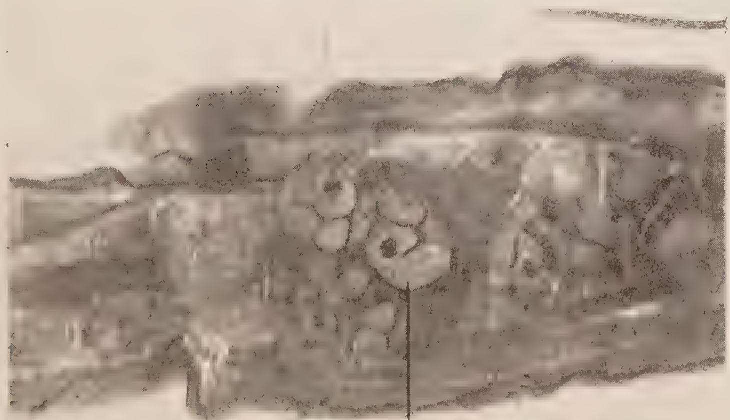

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PLATE XXI

Explanation of Plate XXII

Photographs of median longitudinal sections of pupa.

Fig. 36.—Pseudo-conjugation. At *a* is a sporont transformed for pseudo-conjugation. It is greatly elongated and has a flattened head end. At *b* and *c* can be seen the heads of the two copulae united. This is a very large larva, and the gregarines are proportionally large. × 160.

Fig. 37.—Beginning of cyst formation. At *a* the pair of conjugants has not quite formed the sphere. At *b* this is accomplished. At *c* the large nucleus of each half has divided into numerous parts. × 160.

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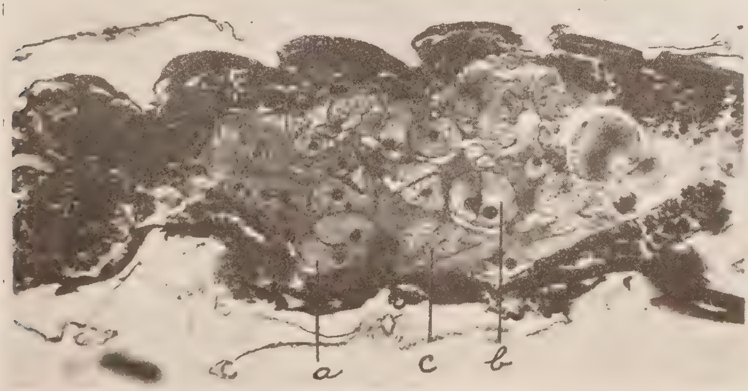

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PLATE XXII

Explanation of Plate XXIII

Fig. 38.—Median longitudinal section of mature female fly with abdomen filled with eggs (*a*). Stomach (*b*) and esophagus (*c*) are filled with spores already free from cyst. × 160.

Fig. 39.—Copulating pair of *Schneideria metamorphosa* showing heads (*a*). × 160.

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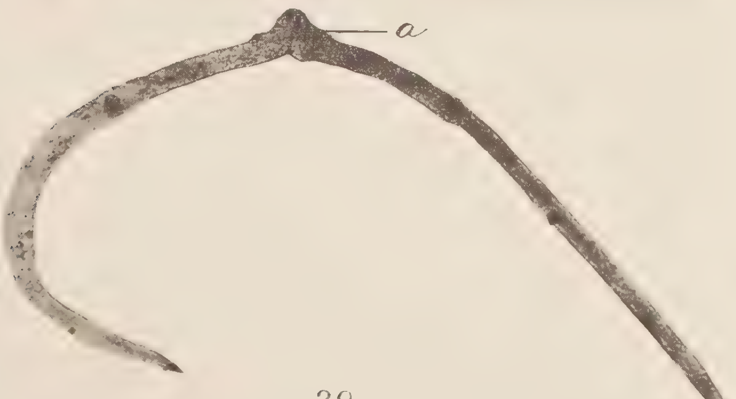

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PLATE XXIII

THE DEVELOPMENT OF *FASCIOLOPSIS BUSKI* LANKESTER

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Fasciolopsis buski is an important human parasite which has been reported from China, India, Assam, Bornea, Sumatra, Cochin China and Tonkin. It is most frequently found in China where it has been reported from Canton, Hongkong, Swatow, Shanghai and other places. According to Goddard (1920) there is a very important endemic center of this parasite in the region of Shaohing in the Chekiang province. In Formosa it is not infrequently found in pigs, but no human cases have as yet been reported.

I obtained specimens of *Fasciolopsis buski* from native pigs in Formosa which measured 25 to 40 mm. in length and 10 to 17 mm. in width. Eggs from these individuals were incubated and free swimming miracidia obtained. Penetration of these miracidia was obtained into *Planorbis coenosus* and the development of the cercaria was studied. Later encysted cercariae were fed to experimental animals and the stages in development to the adult condition were traced. These studies were carried on over a period of five years, and an outline of them has already been published (Nakagawa, 1921). In the present paper I will give a more detailed account of the stages in development.

The development of the eggs of *Fasciolopsis buski* resembles that described by Leuckart for the eggs of *Fasciola hepatica*. The eggs (Fig. 1) fresh from the uteri of the worms are a light yellowish brown in color and measure 0.12 to 0.14 mm. in length (average 0.13 mm.) and 0.07 to 0.09 mm. in width (average 0.08 mm.). A small operculum is present. When these eggs are placed in a small glass dish and the water changed each day, the fertilized egg cell undergoes cleavage and the miracidium soon begins to take form. At first the development is rather slow but later becomes more rapid. During the development of the miracidium the surrounding yolk cells begin to dissolve and gradually disintegrate into what appear to be drops of oil. The end of the developing embryo toward the operculum is broader and develops into the anterior end of the miracidium, while the opposite end early becomes somewhat pointed. As the length of the embryo increases, the surface of its body becomes ciliated and a small protuberance appears at its anterior tip. Just back of this there develops an X-shaped pigmented eyespot. About this time slight extensions and contractions of the body of the embryo can be noted. These movements gradually increase until the miracidium finally pushes

off the operculum and escapes into the water. Figure 2 shows the fully developed miracidium in the egg. The structure of the miracidium (Fig. 3) is very similar to that of the miracidium of *Fasciola hepatica,* but the eyespot is considerably lighter than in that form. The time required for the development of the miracidium varies a little according to the season of the year. In the summer time it generally takes from two to three weeks.

In the summer of 1920 I was able to prove experimentally that two species of snails, viz., *Planorbis coenosus* Benson (Fig. 13) and *Segmentina largillierti* Dkr. (Fig. 14) could serve as the intermediate hosts of *Fasciolopsis buski.* When these species of snails were placed in water containing numerous actively swimming miracidia, the miracidia were soon to be seen swarming around the snails, trying to bore into the head, foot, tentacles, mantle and other parts. As the miracidia penetrated into the snails they threw off their coats of cilia. After penetration they became rounded and immobile, and losing the digestive tract are changed into mother sporocysts. The young mother sporocysts were elliptical in shape and measured 0.08 by 0.07 mm. (Fig. 4). These sporocysts gradually increase in size, the embryonic cells enter into cleavage and gradually rediae become recognizable within the sporocysts (Figs. 5 and 6). The rediae which develop within the sporocysts are cylindrical in shape. In the anterior region of the body, there is developed a ring shaped collar and near the posterior end are formed two lateral protuberances. The pharynx is large and the intestinal cavity is wide containing dark brown material. In the posterior part of the body many germ cells are visible. The redia at this stage of development (Fig. 7) escapes from the mother sporocysts and moves actively. These young rediae penetrate gradually into the walls of the alimentary canal, respiratory cavity and liver of the snail.

As time goes on the rediae increase in size and inside of them develop cercariae. In some of the rediae, however, a new generation of rediae develop. Full grown rediae (Fig. 8) measure from 1 to 1.5 mm. in length and about 0.23 mm. in width. The intestine is now smaller in proportion to the size of the rediae and its contents are deep black in color. The body wall of the redia generally becomes a light brown in color. The mature cercaria while still within the redia measures about 0.23 mm. in length and 0.13 mm. in breadth. It has a long tail which measures 0.4 to 0.5 mm. in length. The body of the cercaria is full of cytogenous glands which give it a dark appearance. At this period a few coarse granules appear near the lower ends of the two divisions of the excretory bladder. Each redia contains from 4 to 7 cercariae. As the cercariae become mature they escape from the redia and swim freely.

The mature cercariae (Fig. 9) have fewer pigment granules than the younger ones. They measure from 0.21 to 0.23 mm. in length and 0.12 to 0.15 mm. in breadth. The tail is long, measuring 2 or 3 times the body length. The cercaria is flattened and has the general shape of a tadpole. The oral sucker is subterminal and has a diameter of about 0.04 mm. The pharynx is spherical, immediately behind the oral sucker, and has a diameter of about 0.02 mm. The bifurcation of the intestinal ceca can be observed close behind the pharynx, but their course is difficult to make out. The divisions of the excretory bladder can be observed in a sinous course on each side of the body. They are very conspicuous on account of the presence of coarse, highly refractile granules, the largest of which have a diameter of 7μ. The undivided portion of the excretory bladder is very small, has an elliptical form which sometimes changes and is not always easy to make out. The ventral sucker is situated a little behind the middle of the body and has a diameter of about 0.03 mm. Soon after their escape from the snail the mature cercariae attach themselves to plants, where they become encysted.

The encysted cercaria (Fig. 10) has a diameter of 0.13 to 0.14 mm. The excretory bladder is filled with coarse granulus, peculiar to this species, which furnish a reliable differential character. The wall of the cyst consists of two layers, and has a thickness of 7μ. Around this cyst is attached some residue from the cercaria which is faintly yellowish. In the summer the cercariae mature in about 5 to 7 weeks after the infection of the intermediate host.

Stages of Development in the Final Host

I fed some dogs and pigs with the encysted cercariae of *Fasciolopsis buski* and studied their development. These experiments are still under way but I will record here the development as far as it has been worked out. Worms obtained from the small intestine of a young pig 20 days after feeding with the encysted stage measured after preservation in formalin 1 to 1.6 mm. in length and 0.48 to 0.58 mm. in width. They were flattened and leaf like in form and light colored. The oral sucker had a diameter of 0.15 mm. and the pharynx 0.12 mm. The ventral sucker was already located in front of the middle of the body and had a diameter of 0.3 mm. The reproductive organs were but slightly developed and could scarcely be distinguished. The surface of the body was thickly set with short spines embedded in the cuticula.

Worms 24 to 27 days after feeding to an experimental puppy measured when preserved in formalin 2 to 3 mm. in length and 1 to 2 mm. in breadth (Fig. 11). In the largest specimen the oral sucker was situated somewhat ventrally and has a diameter of 0.25 mm. Just behind this was the pharynx which was spherical with a diameter

of 0.15 mm. The place of bifurcation of the intestinal ceca was just behind the pharynx, and they ran to the posterior end parallel to the sides of the body. For the posterior half of their length they took a zigzag course. The conspicuous, bell-like ventral sucker is now well forward and has a diameter of 0.6 mm. The reproductive organs were not yet well developed, but the position of the genital pore just in front of the ventral sucker could be made out. The tubular cirrus sac could be traced backward along the median line. The uterus was at this stage a straight tube, the posterior half of which was under the cirrus sac. The shell gland (Mehlis gland) was clearly visible at the posterior end of the uterus. The ovary could be seen as a small group of cells at the side of the shell gland. The testes, also, could be seen as faintly defined groups of cells one behind the other, behind the ovary. The vitellaria were not yet developed at this stage. The main stem of the excretory bladder could be seen to divide into two or three branches just behind the shell gland. The excretory pore was distinctly visible at the posterior tip. The surface of the body appeared to be covered with scales owing to the presence of short spines embedded in the cuticula. The parts of the central nervous system showed clearly on each side of the pharynx.

Worms obtained 25, 28 and 33 days after feeding the cysts to young pigs moved about rather actively in a leech like manner. Some of the more fully extended specimens measured as much as 10 mm. in length. The largest specimens were somewhat reddish in color. They varied in size measuring when preserved in formalin 2 to 5 mm. in length and 1 to 3 mm. in width. They agreed in structure with those obtained from the experimental dog, only some were a little further developed.

Worms obtained from the small intestines of pigs 60, 66 and 89 days after feeding, when preserved in formalin, measured 10, 12 and 18 mm. in length and 4, 5 and 8 mm. in width. The uteri of the larger ones contained many eggs and agreed morphologically with the adult described below. Even in the smaller ones the reproductive organs at this stage were well developed and although the vitellaria were apparently not yet completely developed, the uteri contained eggs.

Worms obtained from experimental pigs 90 and 93 days after the feeding of the encysted cercariae were already fully developed (Fig. 12) and were discharging eggs. When preserved in formalin they were somewhat contracted and measured 13 to 15 mm. in length and 6 to 8 mm. in width. Living specimens were red in color and moved actively with a leech-like movement. Fully extended living specimens measured 20 mm. in length by 10 mm. in width. The oral sucker was 0.4 mm. in diameter and the pharynx, 0.5 mm. The intestinal ceca have the same course as in the descriptions of the adults from man. The bell-shaped ventral sucker is large and conspicuous and measured 1 mm.

in diameter. The genital pore was visible near the anterior margin of the ventral sucker. The cirrus sac was median and long and cylindrical in shape. The ovary and and shell gland are at this stage situated near the middle of the body. The folds of the uterus partly fill the space between the ovary and the ventral sucker and obscure the posterior part of the cirrus sac. The ovary is dendritically branched like the antlers of a stag and is situated to the right of Mehlis' gland. The testes lie one behind the other and are very much branched. The vitellaria occupy the space along the sides of the body from the region of the ventral sucker to the posterior end. The eggs are light yellowish brown in color and measure 0.12 to 0.13 mm. in length and about 0.08 in width. These specimens, although somewhat below the average of the measurements of adult specimens of *Fasciolopsis buski* from man and the pig, agree in structure with the description of this species.

SUMMARY

The miracidia develop and escape from the eggs of *Fasciolopsis buski* in about two to three weeks in the summer time in Formosa. The cercariae are able to develop in two different species of snails, viz., *Planorbis coenosus* Benson and *Segmentina largillierti* Dkr. The most characteristic feature of the cercaria is the conspicuous limbs of the excretory bladder, situated along the sides of the body and filled with coarse granules. The encysted cercariae, which also have this same characteristic, are found attached to water plants. By feeding experimental animals (dogs and pigs) with the encysted cercariae, immature forms and sexually mature adults were obtained from the small intestines.

The life-cycle of *Fasciolopsis buski* closely resembles that of *Fasciola hepatica.* From the eggs which escape into the water, the miracidia hatch which invade the snail host. In the intermediate host the miricidia metamorphose into sporocysts and the development of rediae and cercariae follow. The mature cercariae escape into the water and become encysted on water plants, awaiting ingestion by their final host. The life cycle of *F. buski* as well as its mode of infection furnishes data for the prevention and control of the disease which this parasite produces in man.

In conclusion I wish to express my sincere thanks to Professor Miyajima for his kind assistance.

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EXPLANATION OF PLATE XXIV

Fig. 1.—Underdeveloped egg.

Fig. 2.—Egg containing fully developed miracidium.

Fig. 3.—Miracidium.

Figs. 4-6.—Stages in development of mother sporocyst.

Fig. 7.—Immature redia.

Fig. 8.—Fully developed rediae containing cercariae.

Fig. 9.—Mature cercaria, ventral view.

Fig. 10.—Encysted cercaria.

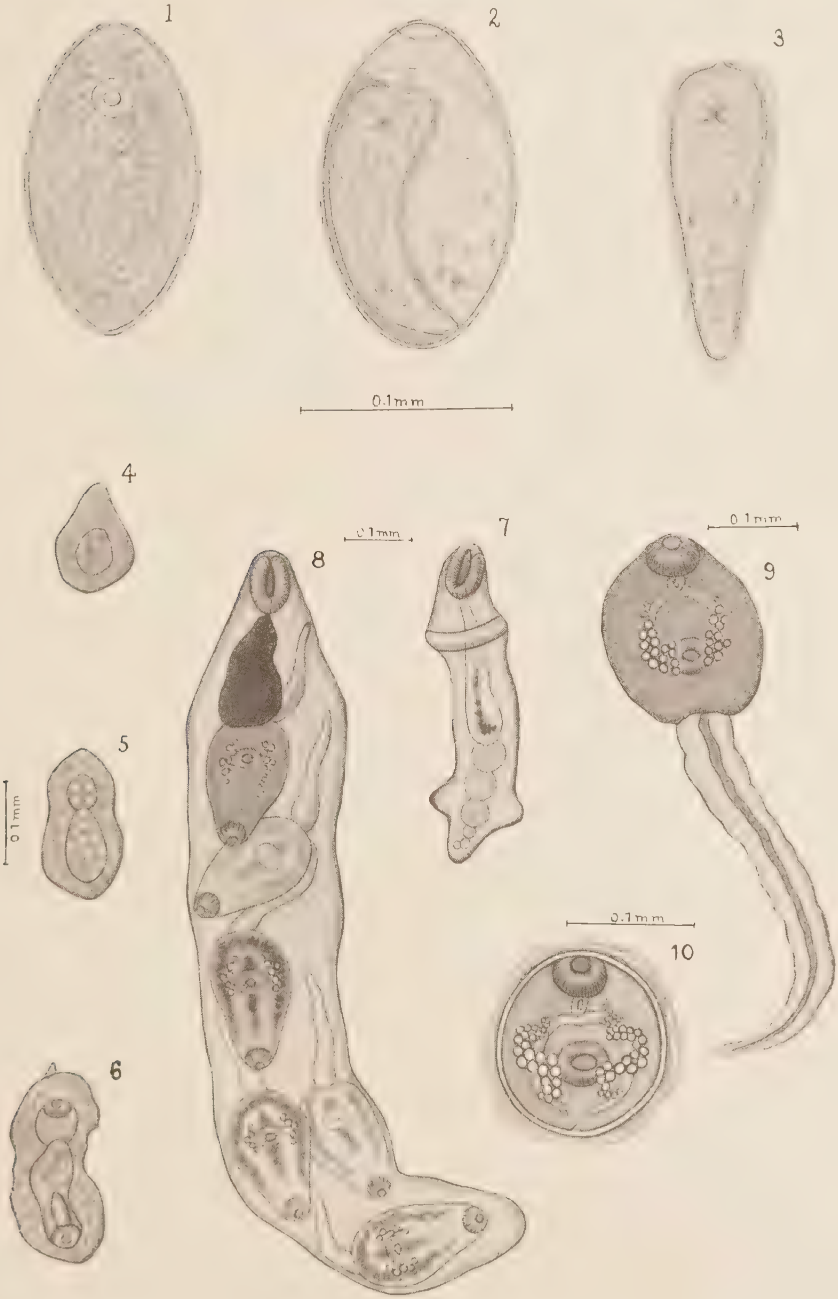

PLATE XXIV

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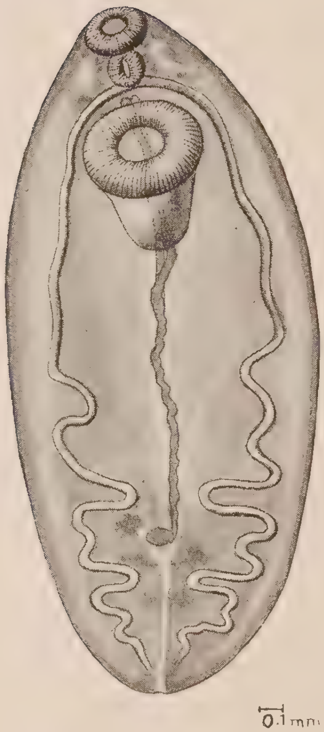

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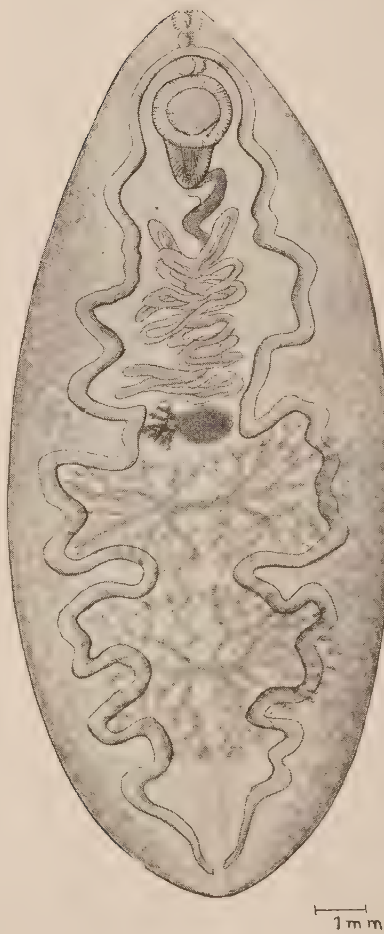

PLATE XXV

Fig. 11.—Immature worm, about 30 days after infection of final host.

Fig. 12.—Sexually mature worm, about 90 days after infection of final host.

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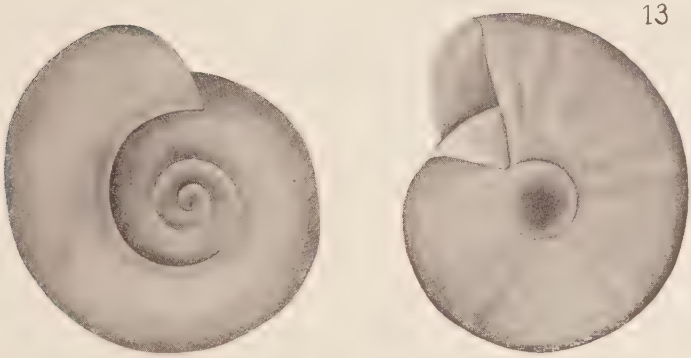

ORIGINAL SIZE

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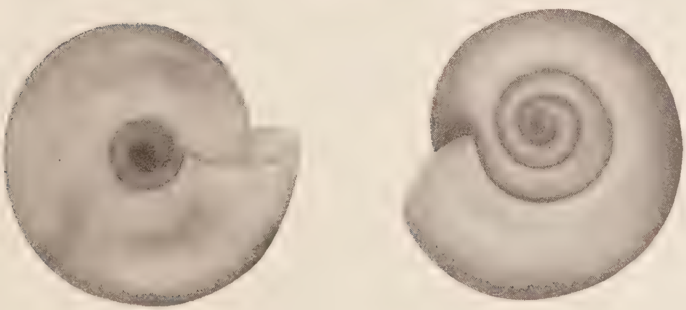

ORIGINAL SIZE

PLATE XXVI

Fig. 13.—*Planorbis caenosus* Benson, intermediate host of *F. buski.* ×5 and natural size.

Fig. 14.—*Segmentina largillierti* Dkr., intermediate host of *F. buski.* ×5 and natural size.

OBSERVATIONS ON THE DEVELOPMENT OF *HETERAKIS PAPILLOSA* BLOCH IN THE CHICKEN

Cesar Uribe

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Heterakis papillosa was first studied by Bloch (1782) who named it *Ascaris papillosa.* In 1791 Fröhlich classified it in the genus Heterakis under the name *Heterakis vesicularis.* Raillet in 1885 accepted Fröhlich's generic denomination and maintained the specific name given by Bloch.

Cecal lesions associated with heterakid worms were first described by Blavette (1840) in chickens infested with *H. perspicillum* (Rudolphi, 1803). Létulle and Marotel (1909) described the lesions produced in pheasants by *H. papillosa,* which had already been noted by Galli-Valerio.

The importance of the parasitism of *Heterakis papillosa* was not realized until Graybill and Smith (1920) found that the disease "blackhead" could be produced in young turkeys by feeding them the embryonated eggs of this worm. This discovery has aroused general interest and has led to further investigations concerning both the life history of this worm and the rôle which it plays in the transmission of blackhead. The manifest economical importance of Heterakis in its relation to the latter disease has led me to undertake some experiments in connection with the development and biology of this worm.

Of recent articles which have appeared on this subject, that of Graybill (1921) on the development of *Heterakis papillosa* in the fowl is of especial interest. The result of my experiments, which were well advanced when this article appeared, confirm for the most part the observations of this writer. As certain additional facts concerning the anatomy and development of the worm will serve to contribute to the general knowledge of its life history it was thought advisable to present the results of my observations. The present paper will deal with the anatomy, development, and biology of the worm as well as with the pathological changes with which it is associated.

Description of the Parasite.—Heterakis papillosa is a small slender worm of a whitish color, tapering at both ends, especially the posterior. The male measures from 7 to 8 mm., the female from 8 to 10 mm. but not infrequently attains a length of 13 to 15 mm. The living worm of either sex, especially immature forms, are often found tightly coiled in rings or spirals. In this case it is rather difficult to distinguish them

in diluted cecal contents. The female often shows a peculiar characteristic curvature, the anterior portion is bent sharply to the dorsal side sometimes making a small loop, while the rest of the body extends in a curve with its convexity to the dorsal side thus giving to the worm the appearance of an elongated interrogation mark. The male may show irregular curvature either to the ventral or to the dorsal side and often shows two or three bends.

The cuticula is smooth and transparent, without transverse striations. Arising from a point near the anterior extremity, two lateral longitudinal ridges are seen which terminate at the posterior extremity in the female in a flattened elongated tail, in the male in a copulatory bursa. These ridges are somewhat of the character of the "wings" of Oxyuris. The anterior extremity is provided with three lips of equal size of which one is dorsal and two ventral. The ventral ones show two small inconspicuous papillae situated at their bases.

The mouth is a simple orifice, followed by a short and narrow oral cavity. On the walls of this cavity and near the oral aperture are three indistinct teeth and at the bottom three other very small teeth are seen, projecting vertically, toward the oral aperture (Figs. 10, 15). The excretory pore is found in the anterior third of the body, slightly posterior to the middle point of the esophagus.

The esophagus, measuring 1 mm., follows the oral cavity described. It is composed of two parts: an anterior portion which is very short and a longer posterior portion. They are separated by a constriction of the walls, showing at this point three small pear-shaped structures with their narrow extremity pointing toward the lumen of the esophagus. The anterior portion of the esophagus, as well as part of the oral cavity, is surrounded by a thickening of the muscular tube, thus forming a definite pharyngeal bulb. The posterior portion possesses a larger dilatation at its posterior extremity. This dilatation or esophageal bulb shows a chitinous structure, which in early stages of development, and in macerated larvae, is found to be composed of two cup-shaped pieces with their expanded portions opposed to one another, and separated by a third flattened intermediate piece (Fig. 9). In the adult this esophageal bulb has the shape of a top with its dilated portion toward the posterior extremity and includes, in addition to the chitinous pieces, some cells which appear to be of a glandular nature. From the anterior extremity of the chitinous bulb, three slender chitinous strip-like processes extend anteriorly along the inner aspect of the muscular esophagus, to the pharyngeal bulb. All these structures are better seen when the worm has been macerated in water for some time.

The intestine extends posteriorly from the pharyngeal bulb as a simple tube with a dilatation at the anterior extremity, which sometimes

is visible to the naked eye as a transparent spot. The gut, which in both sexes extends along the dorsal side of the worm, becomes narrow posteriorly and bends ventrally to terminate in the female in the anal opening which is situated about 1 mm. from the posterior extremity. In the male the gut joins the genital tract to form a cloaca, which opens 0.26 mm. from the tip of the tail.

The female genital apparatus is formed by a double system of ovaries, oviducts, and uteri, which fill most of the remaining space between the intestine and the body wall. The ovaries are followed by the oviducts, which at their junction with the uteri show an elongated receptaculum seminis. The vagina consists of a muscular tube, of which the portion immediately following the junction of uteri serves as an ovijector. The vagina opens at the genital pore a little posterior to the middle portion of the body. The arrangement of the terminal portion of the female reproductive system is shown in figure 22.

In the male the copulatory bursa is formed by the posterior expansion of the lateral wings, supported by papilliform rays, and is followed by a short tail. Twelve papillae are distributed on each side of the caudal extremity as follows: three around the sucker, one anterior, one lateral and a third near the anus; five adanal, two toward the posterior extremity, and the remaining two lying between these groups (Fig. 14). The sucker, which measures about 60μ in diameter, is situated anteriorly to the anal opening and is provided with a circular chitinous rim exhibiting a papilliform nodule in its posterior border. At its base it possesses a glandular structure (Fig. 27) which secretes a sticky substance.

The spicules are unequal in size, the shorter one measuring about 0.65 mm. in length or approximately one-third that of the longer one which is 2 mm. long. They are enclosed in separate sheaths which arise one on each side of the intestine but posteriorly pass to the dorsal side and, following the curvature of the terminal portion of the gut, bend ventrally to coalesce at the outlet of the cloaca. The shorter spicule is tubular and possesses two lateral flanges (Figs. 13, 13a). The tip has a characteristic twist and is often protruded outside of the body (Fig. 12). The longer spicule is also tubular but possesses only one lateral flange (Figs. 13a, 25). The tip is sharp and straight and is often hidden inside of the body.

The male reproductive system shows a single tubular testis followed by the spermatic duct which terminates in an elongated dilatation or seminal vesicle. The seminal vesicle is followed by a muscular duct which extends along the ventral side of the last portion of the intestine and, like the latter, opens into the cloaca (Figs. 27). In its last portion it possesses some ventrally situated cells which seem to be of a glandular character.

The freshly laid egg is symmetrical, smooth, ovoid, and unsegmented. According to measurements it is 68 to 75μ in length by 36 to 38μ in width. It possesses a thick shell, showing a thickening at one of its extremities in which a lenticular clear space is sometimes seen. The contents of the egg appears as a dull yellowish granular material, which together with the enclosed nucleus fills the egg completely.

Its development outside the host is greatly influenced by environment and it is apparently very resistant to unfavorable influences. In the experiments I used worms from different lots of chickens and turkeys. The worms were washed in several changes of sterile salt solution, and chopped into small bits, which were kept moist at room temperature in Petri dishes. Under these conditions the ova were embryonated in from 9 to 12 days. Some lots, kept in wide-mouthed test tubes under a rather high column of fluid, were found to take about three weeks to mature and at this time a large percentage of the eggs were usually undeveloped and others were disintegrating. A small amount of moisture appears to be more favorable for development than a large volume of fluid. Heavy growths of bacteria and fungi also seem to interfere with development, retarding it and causing many of the eggs to disintegrate. The large number of amoebae which have appeared in practically every culture does not seem to have any effect on their growth.

Unsegmented eggs failed to develop when placed in the ice-box for several days but proceeded to do so when transferred to room temperature. Fully mature eggs were exposed out of doors in a Petri dish to the alternate freezing and thawing of winter weather from Dec. 9, 1921, to Jan. 20, 1922, when some showed larvae still alive.

Embryonated eggs were placed in the hollow of hanging drop slides, allowed to dry at room temperature, and were subsequently examined at intervals of 24 hours after adding a small quantity of salt solution. It was found that after the ninth day the larvae, when freed from the egg shell, were motionless and easily broken. Some showed a folding of the cuticula. By the twelfth day all of them were motionless, granular, flattened, and apparently dead.

The resistance of non-embryonated eggs to various antiseptic solutions of different concentrations was tested according to the procedure of Yoshida (1920). In this experiment the following chemicals were used in the concentrations indicated: Hydrochloric acid, 10 and 15 per cent.; sulphuric acid, 10 and 12 per cent.; nitric acid, 1 and 1.5 per cent.; corrosive sublimate, 1 and 1.5 per cent.; formalin, 10 and 15 per cent.; acetic acid, 10 and 12 per cent.; phenol, 0.5 and 0.6 per cent. In phenol and hydrochloric, sulphuric, and acetic acid the eggs did not develop at all, some were promptly destroyed, others showed vacuoliza-

tion of their contents after two weeks of exposure. Eggs put into corrosive sublimate were soon destroyed, while some of those in formalin began to develop, but a large percentage, however, soon showed degenerative changes and all failed to attain complete development. In 1.5 per cent. nitric acid many eggs developed after three weeks and were kept alive in this solution for four months (March 9 to September 23). These eggs were eventually all used in feeding experiments, but another lot of eggs have remained alive in 1.5 per cent. nitric acid for seven months (May 19 to Dec. 29, 1921).

Development of the Larva

Different lots of chickens and Heterakis material from various sources were used in the study of the development of this worm in the host. The first lot of chickens when two days old were placed in table-brooder and fed mature Heterakis eggs collected two months earlier. Chicks were then killed at intervals of twenty-four hours during the first days of the experiment and the intervals lengthened thereafter. By carefully examining the cecal contents and scrapings of the mucosa successive stages in the development of the larvae were obtained. The liver and lungs were examined in the fresh state by comminuting the tissue, shaking in salt solution, centrifugating and examining the sediment. After a week from the time of feeding the worm eggs attention was confined to the walls of the cecum and its contents, and both scrapings of the mucosa and paraffin sections of the organ were utilized for study. Sections of other organs were made whenever anything suspicious was detected on gross examination. For the study of the later stages older chickens, kept isolated in order to avoid any additional contamination, were used on different occasions. As a more or less standardized procedure was established in these examinations, the observations will be summarized without detail as to the technique and the conditions of each experiment.

The larva when hatched or freed by cracking the egg shell is colorless. It shows the beginning of the intestinal tract as a granular band extending from the anterior portion, a little posterior to its extremity, to the anus, which is already visible (Fig. 3). According to Graybill, the eggs hatch in the small intestine. They then evidently pass with its contents to the large intestine, from which they enter the ceca, where all the subsequent changes of their development are to be observed. Larvae are found in the cecal contents 24 hours after feeding the eggs. Neither at this time nor subsequently was it possible to demonstrate larvae in other organs. At this stage they measure 180μ, but the morphology of the intestinal tract is, as yet, unchanged. The mouth is clearly visible and shows a subterminal position, as though the

dorsal border were a little longer than the ventral. The pharyngeal bulb is undeveloped. The rate of growth in the days that follow is shown in the accompanying diagram (Chart 1).

The small worms now evidently migrate into the cecal glands, for from the second to the fifth day no larvae were found in the cecal contents, although many were present in the scrapings from the mucosa. Up to the fourth day larvae were found in stained sections in the bottom of the crypts of the cecal mucosa (Fig. 29). Subsequent development takes place chiefly outside of the glands in the lumen of the cecum. By the ninth day the rate of growth shows a sudden

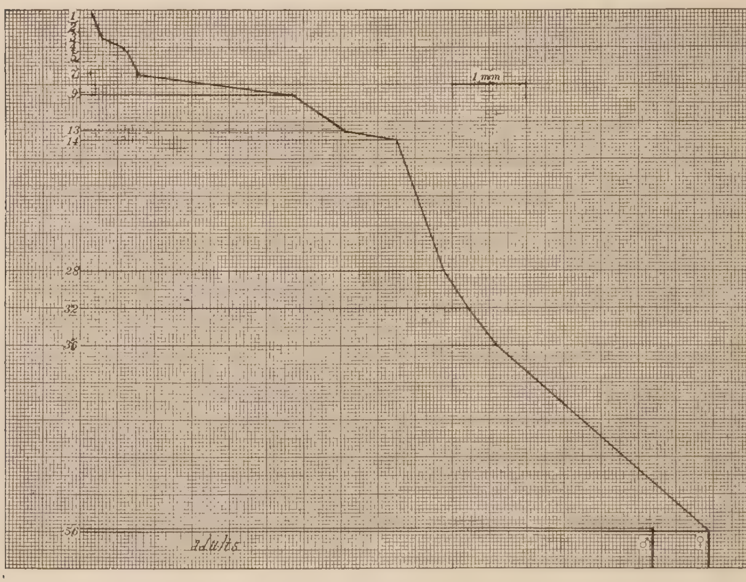

Chart I

acceleration (see diagram) and moulting individuals were found. At this stage the mouth is terminal and the posterior extremity shows characteristic differentiation of the male and female. The female exhibits a group of cells in its middle portion, corresponding to the genital organs. At this stage the worms begin to cause slight damage to the epithelium of the cecal glands, into which their anterior third is often found inserted.

On the eleventh day there are several coils of the genital apparatus apparent at the middle of the body. The pharynx shows the beginning formation of the chitinous pieces seen in the adult. At this stage sections of the cecum showed slight but definite lesions, consisting of erosion of the epithelium and minute hemorrhages. Very little or no

reaction was found in the adjacent tissues. On the thirteenth day the mouth shows three distinct lips and the pharyngeal bulb has the chitinous apparatus characteristic of the adult, although it is not completely developed (Fig. 9). The female genital pore is readily visible as well as a muscular vagina and several coils of the genital canal (Fig. 11). The excretory pore also is already formed.

One of the chickens used in these experiments contracted blackhead, and when killed thirteen days after the ingestion of Heterakis ova, showed firm, large, hemorrhagic cores, ulcerations of the ceca and typical liver lesions, similar in character to those found in turkeys. Many worms were found in the glandular pits and some embedded in the mucosa (Figs. 30-32).

Later stages of development show nothing of particular interest. As already noted, the worm is subsequently, for the most part, found free in the cecal contents. The genital organs develop slowly. Females with eggs which were found 56 and 61 days after the ingestion of ova are considered to represent the completed adult stage. At this stage the worms begin to lay eggs which are passed in the cecal discharges. Although immature females were found up to the forty-eighth day, these may have been derived from the re-ingestion of eggs that had passed through the alimentary tract without hatching. The data at hand are insufficient to definitely establish the exact period necessary for the completion of development.

Disintegrating adult females were sometimes found in the last portion of the large intestine. The ingestion of fecal material contaminated with one of these dead worms would undoubtedly result in a severe infestation in case sufficient time has elapsed for the eggs to become embryonated.

Habitat and Pathology

Heterakis papillosa is found in the ceca of various birds, and especially of the common fowl. It appears to live free in the intestinal contents, although in some instances adult worms were found lightly attached to the mucosa by their anterior end. When loosened, small inconspicuous holes were left in the mucosa at the points of attachment (Fig. 28); similar depressions were observed in sections of the mucosa. In the course of the examination of the ceca of a large number of chickens and turkeys the worms were occasionally found, in part, included in the cavities of the lymph nodules which in such instances were much enlarged. This may be regarded as an abnormal or accidental relationship since it is not of common occurrence. The reported encystment of Heterakis in the wall of the ceca (Létulle and Marotel, 1909) also represents apparently an unusual relationship of host and parasite.

While the adult forms appear for the most part free in the cecal contents, the younger stages up to the fourth day occur in the glands of the mucosa. Stained sections show also in many instances the head of larger but immature worms buried in the mucosa. The character of the mouth of the adult worm with its peculiarly formed teeth suggests that it may attach itself to the wall of the cecum and accordingly feed upon tissue fluids. A number of worms examined have failed to give the iron reaction. That the worms may, under certain conditions, ingest blood is conclusively shown by one case in which all the worms (about 30) taken from the ceca of a hen, showed a visible red spot at the anterior dilatation of the intestine. Stained sections of these worms showed nucleated red corpuscles in the oral cavity and esophagus. The intestinal contents contained a considerable amount of pigment in addition to masses of bacteria. Unfortunately the fixative used for these worms was not favorable for the demonstration of iron. It should be taken into account, however, that the hen from which these worms were taken was distinctly abnormal. No food had been taken for several days and on postmortem examination a generalized peritonitis was found, probably originating from the oviduct. The ceca were almost empty but showed no lesions.

Even in cases of experimental infestation the pathological changes may consist of no more than a slight flattening of the epithelium in contact with the worm and associated foci of infiltration with eosinophiles. The epithelium may be eroded to some extent with the production of small areas of hemorrhage but the injuries are so minute that they are found only on careful study of stained sections. In a few of the cases sectioned the larvae were found embedded in the tissues but here also the reaction was very slight. In one case showing large numbers of worms either embedded in the mucosa or with the anterior extremity extending into the gland pits, the reaction was masked by a concomitant infection with *Histomonas meleagridis* (Figs. 30-32).

In later experiments nineteen newly hatched chicks were fed with material containing embryonated Heterakis eggs and killed 21 days later.

A large proportion of these chickens developed a typhlitis, the lesions having the general appearance and distribution of the cecal lesions in "blackhead." Nine of these chickens showed thickening of the cecal mucosa, cores and healed ulcerations, but the organism of "blackhead" was not demonstrable in stained sections of any of the lesions.

In experiments carried out by Smith and Graybill (1920) a large percentage of brooder chickens which were fed Heterakis ova developed blackhead, the disease in all cases being limited to the ceca. The black-

head organism was demonstrated in active lesions from the ninth to the fifteenth day after the ingestion of Heterakis ova but older lesions showed no parasites. Since all the chickens in our experiment were killed 21 days after infestation with Heterakis, it is probable that the lesions found represent a late stage of a transient blackhead infection.

SUMMARY

The present study serves to establish certain hitherto unrecognized anatomical features of *Heterakis papillosa*. The observations of Graybill concerning the presence of chitinous teeth about the mouth are confirmed. A muscular thickening of the nature of a pharyngeal bulb is described and the position of the excretory pore is fixed at a point about opposite the middle of the esophagus.

Findings regarding the spicules differ from those of Lauro Travassos (1913) for the same species studied in Brazil. The spicules are described by this author as being equal and only 0.27 mm. long. The spicules in my specimens are not only unequal in size, one being about three times as long as the other, but they are quite different in shape. The shorter one, 650μ, shows two lateral flanges and a characteristic twist of its tip, while the longer one, 2 mm., has only a single flange and shows a uniform curvature.

The results obtained with respect to the resistance of Heterakis ova to drying and to variation of temperature for the most part confirm those of Graybill. The resistance of these eggs to chemicals in solutions of various strengths has also been determined. The fact that they develop uninjured in 1.5 per cent. nitric acid which renders the material external to the shell of the egg bacteriologically sterile, may be of value in determining the source of the protozoon of blackhead.

Heterakis papillosa after hatching in the intestine of the fowl undergoes its further development in the ceca. In no instance were larval worms found in other organs or other portions of the body. Most of the larvae throughout the first stages of their development, that is for the first five days, are to be found buried in the glands of the cecal mucosa. As they increase in size many show the anterior extremity inserted in the mucosa but the mature worms usually are free in the lumen of the cecum. It is apparent from the measurement of a considerable number of worms that the rate of growth is accelerated after each moult. Since females containing eggs were not found until after eight weeks from the time that the eggs were fed, a considerable period of time is apparently required for this species to mature. Not only are the eggs passed in the cecal discharge, but occasionally dead females are also found. After sufficient incubation for the eggs to ripen, such material should produce heavy infestation on ingestion by a suitable host.

Although the adult stage of *Heterakis papillosa* lives in the lumen of the cecum, it is not rare to find these worms with the head buried in the mucosa and probably in rare instances the entire worm may be buried in the cavity of the lymph nodules which occur at intervals in the wall of the cecum. It is probable that the adult worms usually feed upon the cecal contents, but all those derived from a certain case showed blood in the alimentary tract. This may have been the result of unusual circumstances, for the hen from which they were derived had eaten nothing for several days prior to its death and showed a peritonitis at postmortem. The structure of the mouth and the possession of definite teeth suggest the possibility of attachment and the utilization of tissue fluids as food.

No evidence has been obtained that this parasite produces very serious injury to the cecal mucosa of the chicken. As the result of microscopical study it was found that the epithelium in contact with the surface of the worm might be stretched and flattened or in some instances slightly eroded. Small areas of hemorrhage and foci of infiltration with eosinophiles were noted in few instances. In a series of chickens that were not killed until 20 days after the ingestion of Heterakis ova a large proportion showed a marked typhlitis. It is not improbable that this condition represented a late stage of a transient blackhead infection such as that described by Smith and Graybill. At least no protozoa were demonstrable in any of the lesions. A chicken of another series, killed on the thirteenth day, showed blackhead with typical lesions of both liver and cecum. Whether Heterakis produces greater injury in the cecum of the turkey than in that of the chicken is not at present known.

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Explanation of Figures

The camera lucida was employed in all except Figure 28.

Explanation of Plate XXVII

Fig. 1.—Unsegmented egg. × 120.

Fig. 2.—Embryonated egg. × 120.

Fig. 3.—Larva free from shell. × 120.

Fig. 4.—Larva in cecum of chicken 24 hours after feeding eggs. ×120.

Fig. 5.—Larva four days old showing subterminal mouth. × 120.

Fig. 6.—Larva five days old. Central spot marking beginning of reproductive system. × 120.

Fig. 7.—Larva six days old. Mouth still subterminal; esophagus clearly differentiated. ×120.

Fig. 8.—Anterior extremity of larva nine days old; mouth terminal. × 120.

Fig. 9.—Anterior extremity of larva 13 days old. × 120.

Fig. 10.—Anterior extremity of adult showing papillae, oral cavity, and teeth. × 220.

Fig. 11.—Genital apparatus of female 12 days old. × 120.

Fig. 12.—Profile of posterior extremity of male showing comparative size of two spicules. ×100.

Fig. 13.—Short spicule with two flanges. ×100.

Fig. 13 a.—Cross section of spicules near cloaca showing long spicule with one flange and short spicule with two included in the same sheath. ×220.

Fig. 14.—Front view of posterior extremity of male showing circular sucker and arrangement of the papillae. × 100.

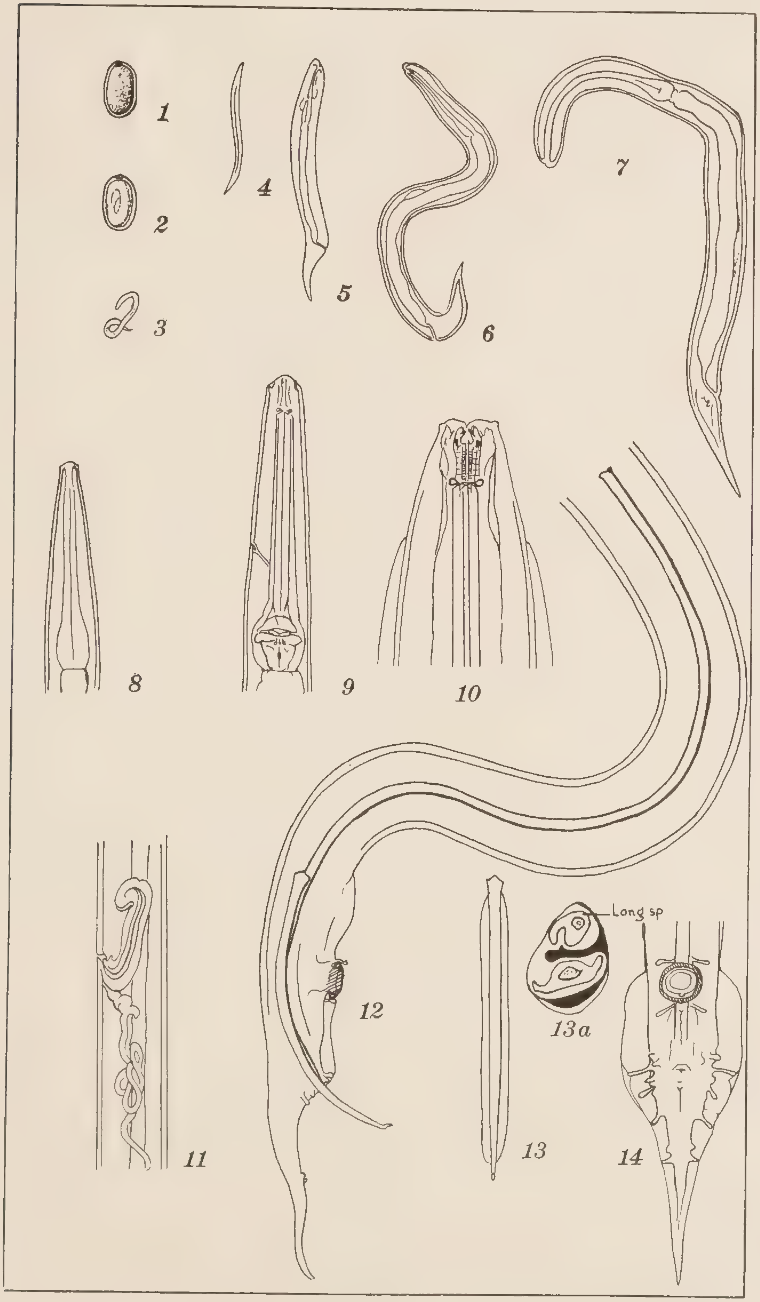

PLATE XXVII

Explanation of Plate XXVIII

Fig. 15.—Stained section of anterior extremity of adult worm showing mouth cavity with teeth, surrounded by pharyngeal bulb. × 60.

Fig. 16.—Longitudinal section of esophageal bulb and of intestinal dilatation. × 60.

Figs. 17, 18 and 19.—Cross sections of esophagus at different levels. × 60.

Fig. 20.—Cross section of female showing intestine, ovary, and uterus. × 60.

Fig. 21.—Cross section of female near genital pore. ×60.

Fig. 22.—Longitudinal section of female showing ovary, uterus, receptaculum seminis, vagina and genital pore. × 60.

Fig. 23.—Cross section of female showing intestine, ovary, receptaculum seminis, and uterus. × 60.

Fig. 24.—Cross section of male showing intestine, testes and long spicule. × 60.

Fig. 25.—Cross section of male showing intestine, both spicules, testes, and vas deferens. × 60.

Fig. 26.—Cross section of spicules at cloaca. × 60.

Fig. 27.—Longitudinal section of male posterior extremity showing vesicula seminalis, intestine, glandular structures and spicule. × 60.

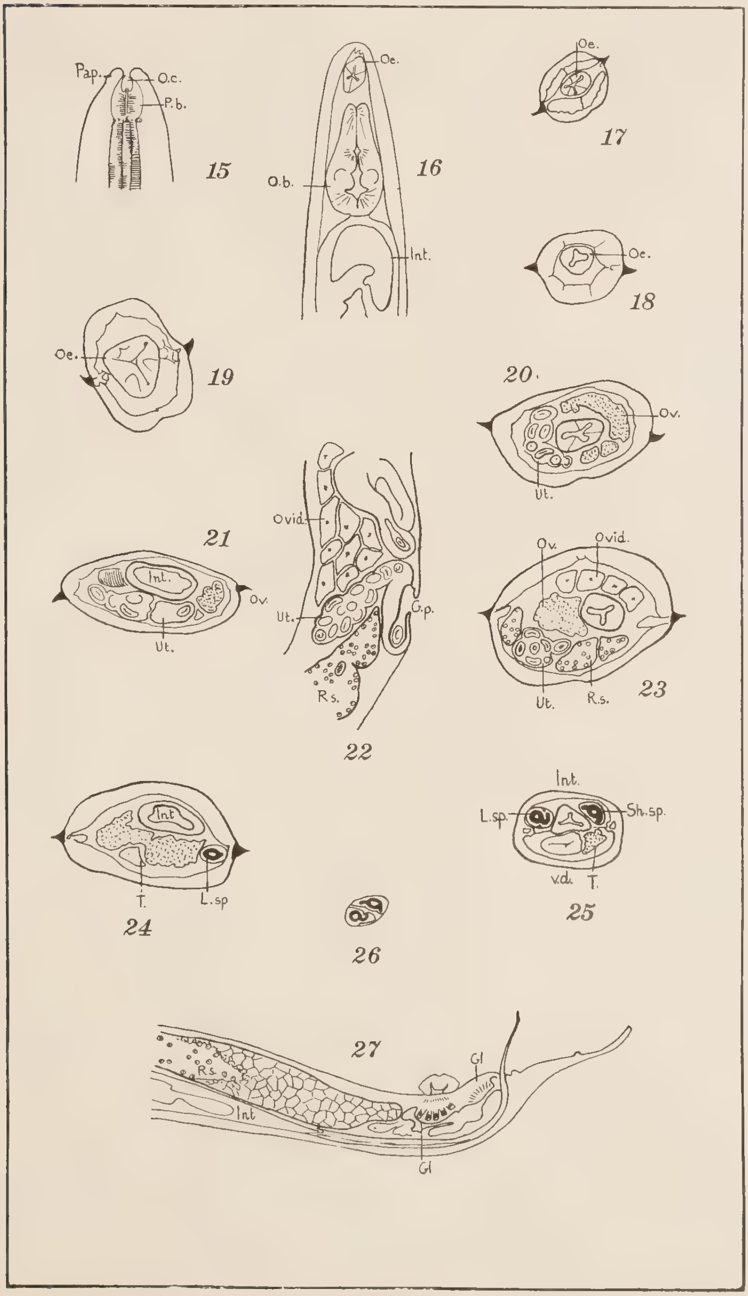

PLATE XXVIII

Explanation of Plate XXIX

Fig. 28.—Drawing of worm partially included in lymph nodule which shows enlargement. $\times 3$.

Figs. 29-32.—Microphotographs taken at the same magnification. Four day old larvae in gland of cecal mucosa.

Fig. 30.—Section showing larva embedded beneath the mucosa. Blackhead parasites present.

Fig. 31.—Anterior extremity of larva inserted in glandular crypt; numerous blackhead parasites apparent.

Fig 32.—Cross section of larva embedded in tissue and surrounded by blackhead parasites.

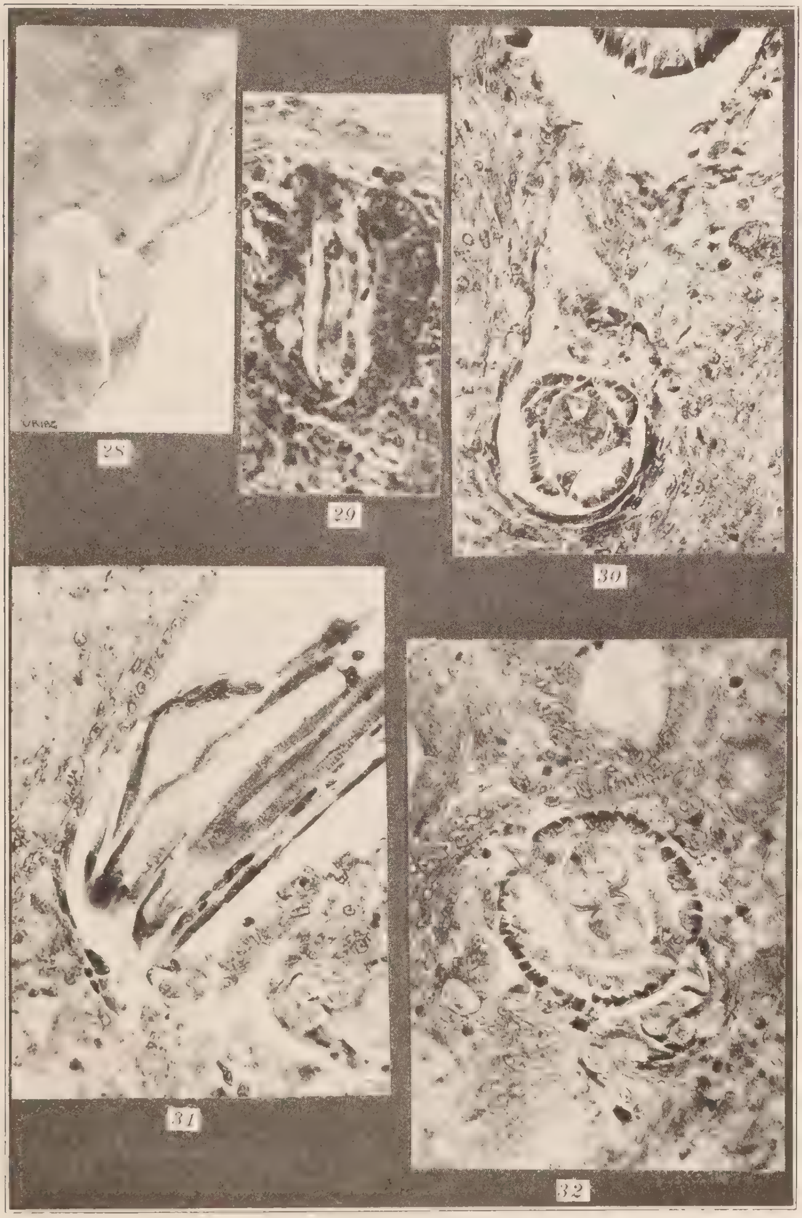

PLATE XXIX

A STUDY OF THE ESCAPE OF CERCARIAE FROM THEIR SNAIL HOSTS *

William W. Cort

During the summer of 1920 while studying at the University of Michigan Biological Station at Douglas Lake, Michigan, I undertook a series of experiments on the escape of cercariae from their snail hosts. The object of these experiments was to determine the numbers of cercariae escaping from snails infested with trematodes and the times at which these cercariae made their escape. Four species of cercariae were studied, viz.: (1) *Cercaria elephantis* Cort (1917), a schistosome cercaria with eyespots from *Planorbis trivolvis* Say; (2) *Cercaria emarginatae* Cort (1917), a forked tailed cercaria with a pharynx from *Lymnaea emarginata angulata* Sowerby; (3) an undetermined echinostome cercaria from *Physa ancillaria parkeri* Currier, and (4) an undetermined stylet cercaria from the same host. Most of the studies were made on the escape of *Cercaria elephantis* from *Planorbis trivolvis,* the small number of experiments on the other three species of cercariae being carried out near the end of the work to obtain some comparative data.

In order to determine which snails were infested with the cercariae the following simple method was used: A collection of about 100 specimens of a given species of snail would be brought into the laboratory late in the afternoon. These snails were then divided into groups of four or five and the groups placed in separate wide mouthed six or eight ounce bottles about one-third full of water. The next morning some of the water from these bottles was examined in a watch glass under a dissecting microscope. If any cercariae had escaped they could be easily seen and the groups containing infested snails determined. These groups were then redivided, only one snail being placed in a bottle, and by further examinations the individual snails infested were determined. Examination of the water from negative groups was repeated late in the afternoon to catch any cercariae which escaped only in the daytime. When a high percentage of snails were infested no preliminary division was made, the snails being immediately placed in separate bottles.

With the infested snails isolated it was possible to pour off the water from around them at definite intervals and count the cercariae given off during certain definite periods. A new supply of water was

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* A contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University and from the University of Michigan Biological Station.

TABLE 1.—Data on the Escape of Cercaria elephantis from Planorbis trivolvis

Day	Hour	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
July													
4	11 a m - 3:30 p m	146	0	0									
4	3:30 p m - 9 p m	5	420	121									
4-5	9 p m - 8 a m	3	*	*									
5	8 a m - 1 p m	1000±	8	4									
5	1 p m - 7 p m	186	104	25									
5-6	7 p m - 9 a m	5	150	164									
6	9 a m - 12 n	43	3	3									
6	12 n - 3 p m	219	0	0									
6	3 p m - 6 p m	19	0	0	43	4	47						
6	6 p m - 9 p m	5	6	1	7	0	3						
6-7	9 p m - 7 a m	5	129	5	15	60	6						
7	7 a m - 9 a m	20	26	3	134	11	21						
7	9 a m - 12 n	653	2	1	127	1	150						
7	12 n - 3 p m	167	3	0	70	0	85						
7	3 p m - 5 p m	32	33	2	23	6	61						
7	5 p m - 7 p m	4	254	3	1	52	71						
7	7 p m - 9 p m	15	610	161	2	166	4						
7-8	9 p m - 7:30 a m	1	295	125	75	125	43						
8	7:30 a m - 9 a m	720	25	27	220	18	113						
8	9 a m - 12 n	882	7	6	180	5	95						
8	12 n - 3 p m	450	2	0	104	0	93						
8	3 p m - 6 p m	41	60	0	77	0	61						
8	6 p m - 9 p m	34	296	135	12	64	16						
8-9	9 p m - 12:30 a m	1	392	46	12	70	7						
9	12:30 a m - 3:15 a m	0	40	12	0	24	1						
9	3:15 a m - 6:45 a m	0	14	2	1	5	0						
9	6:45 a m - 9 a m	55	2	3	211	1	82						
9	9 a m - 12 n	490	0	2	213	0	108						
9	12 n - 3 p m	797	0	0	98	1	110						
9	3 p m - 6 p m	50	30	1	37	0	82						
9	6 p m - 9 p m	11	489	247	7	31	6						
9-10	9 p m - 12:15 a m	0	146	46	1	40	2						
10	12:15 a m - 3:30 a m	0	4	75	0	21	0						
10	3:30 a m - 6:45 a m	0	9	5	0	0	0						
10	6:45 a m - 9 a m	75	4	2	140	0	14						
10	9 a m - 12 n	1218	0	3	111	0	114						
10	12 n - 3 p m	559	2	1	346	0	47						
10	3 p m - 6 p m	35	186	60	76	5	85						
15	5:30 p m - 9 p m	....	82	6	5	....	....	75	35	42	0	1	1
15	9 p m - 12 m	....	36	23	2	....	....	12	10	32	0	0	0
16	12 m - 3 a m	....	11	7	0	....	....	0	0	0	0	0	0
16	3 a m - 6 a m	....	3	1	2	....	....	0	0	0	1	0	0
16	6 a m - 9 a m	....	1	1	7	....	....	0	0	0	0	1	0
16	9 a m - 12 n	....	5	1	120	....	....	1	0	0	0	43	7
16	12 n - 3 p m	....	10	1	61	....	....	2	0	0	0	91	25
16	3 p m - 6 p m	....	151	44	31	....	....	61	18	38			
16	6 p m - 9 p m	....	110	226	13	....	....	307	269	565	....	3	4
16	9 p m --12 m	....	24	154	0	....	....	10	34	2	....	0	0
17	12 m - 3 a m	....	4	40	0	....	....	2	11	0	....	0	0
17	3 a m - 6 a m	....	5	6	1	....	....	....	5	....	....	0	0
17	6 a m - 9 a m	....	....	....	143	....	....	....	....	....	....	27	20
17	9 a m - 12 n	....	....	....	98	....	....	....	....	....	....	56	37
17	12 n - 3 p m	....	....	....	25	....	....	....	....	....	....	49	82
17	3 p m - 6 p m	....	....	....	57	....	....	....	....	....	....	15	5
19	7:30 a m - 9 a m	....	....	....	112	....	....	....	....	....	....	62	
19	9 a m - 12 n	....	....	....	119	....	....	....	....	....	....	90	
19	12 n - 1 p m	....	....	....	1	....	....	....	....	....	....	70	
19	1 p m - 6 p m	....	....	1	....	....	....	....	....	....	1		
19	6 p m - 9 p m	....	....	1	....	....	....	....	....	....	114		
19	9 p m - 12 m	....	....	6	....	....	....	....	....	....	145		
20	12 m - 7:30 a m	....	....	11	....	....	....	....	....	....	5		
Aug.													
5	9 a m - 12 n	....	....	0	15	....	....	....	....	3	....	12	
5	12 n - 3 p m	....	....	0	29	....	....	....	....	1	....	6	
5	3 p m - 6 p m	....	....	0	30	....	....	....	....	2	....	5	
5	6 p m - 9 p m	....	....	0	1	....	....	....	....	4	....	0	
5	9 p m - 12 m	....	....	2	0	....	....	....	....	7	....	0	
6	12 m - 3 a m	....	....	5	0	....	....	....	....	0	....	1	
6	3 a m - 6 a m	....	....	6	0	....	....	....	....	0	....	0	
6	6 a m - 9 a m	....	....	0	9	....	....	....	....	1	....	2	
6	9 a m - 12 n	....	....	0	17	....	....	....	....	3	....	7	
6	12 n - 3 p m	....	....	0	21	....	....	....	....	1	....	8	
6	3 p m - 6 p m	....	....	0	6	....	....	....	....	1	....	1	
6	6 p m - 9 p m	....	....	15	9	....	....	....	....	z	1		
6-7	9 p m - 7:30 a m	....	....	14	7	....	....	....	....	9	0		

* Not counted.

then poured into the bottles and the process repeated as desired. Of course it is possible that all cercariae free in the water would not be poured out, but the bottles were thoroughly rinsed each time the water was poured off and in all cases the treatment was the same, so that this hardly seems to be an important source of error. For counting enough formalin was mixed with the water to kill the cercariae and they were counted in a ruled Syracuse watch glass under a dissecting microscope. By this method it can be determined how many cercariae escape from a particular snail during a given period and whether they escape at regular intervals or in cycles.

The Numbers of Cercariae Escaping from Snails

The data obtained in regard to the escape of *Cercaria elephantis* from *Planorbis trivolvis* are included in Table 1. On examining this table, one is struck with the large numbers of cercariae which must escape from an infested snail of this species during the course of a summer. The data do not give accurate information on this point, since the length of the period of cercaria-production in any one snail is not known. The experiments give records of cercariae escaping from snails III and IV for periods extending over about a month. The record is more complete for snail IV, from which cercariae escaped as follows: (See Table 1)—July 7, 372; July 8, 680; July 9, 568; July 10, 673; July 16, 234; July 17, 324; July 21, 447; July 22, 263; August 6, 69. The small number of cercariae escaping from this snail on August 6 may indicate that the period of cercaria-production was nearing its end. This conclusion does not necessarily follow, however, since snail IV by August 6, was in an advanced stage of starvation, which may have influenced the number of cercariae produced. These nine records which are scattered through the month give an average per day of 400 cercariae escaping. For the period of 31 days, during which this snail was observed, this would give a total of about 12,400 cercariae, which may be taken as an approximation of the number of cercariae which escaped from this snail during the month. A single month certainly does not represent the total period of cercaria production in this snail, since the number was at its height when the observations started.

Further, in some of the other snails the numbers of cercariae escaping each twenty-four hours were much larger, as for example snails I and II (see tables 1 and 3). These figures give some idea of the enormous numbers of cercariae of this species which would escape into a body of water containing only a relatively small number of infested individuals of *Planorbis trivolvis*.

When the records of the escape from their snail hosts of the other three species of cercariae studied are examined, the numbers involved

are even more striking (see Table 2). For example, in the case of snail IV of the stylet cercaria series over 3,000 cercariae escaped on August 14, and in the *Cercaria emarginatae* series over 5,000 cercariae escaped from snail V on August 16 (see Table 2). These findings emphasize the enormous reproductive wastage in the development of cercariae in the digenetic trematodes, which is necessary on account of the great difficulty that they experience in reaching their final hosts.

TABLE 2.—Data on Escape from Snail Hosts of Three Cercariae

Day Aug.	Hour	Echinostome Cercaria				Stylet Cercaria				Cercaria emarginatae					
		I	II	III	IV	I	II	III	IV	I	II	III	IV	V	VI
12	10 a m - 2 p m	446	535	251	233	272	350	673	628	18	0	148	425		
12	2 p m - 6 p m	157	200	177	275	242	270	324	430	234	3	67	475		
12	6 p m - 10 p m	30	12	5	17	414	269	244	1061	213	6	142	28		
12-13	10 p m - 6 a m	17	1	3	0	255	309	765	377	95	166	664	14		
13	6 a m - 10 a m	189	12	4	5	230	407	822	449	192	18	211	266		
14	5:15 a m - 8:15 a m	...	49	...	...	...	...	...	1020	...	...	...	65		
14	8:15 a m - 11:15 a m	...	55	...	...	...	...	...	840	...	...	...	165		
14	11:15 a m - 2:15 p m	...	249	...	...	...	...	...	1323	...	...	...	109		
14	2:15 p m - 5:15 p m	...	116	...	...	...	...	...	687	...	...	...	277		
14	5:15 p m - 9:45 p m	...	153	...	...	...	...	...	490	...	...	...	146		
14-15	9:45 p m - 9 a m	...	1	...	...	...	...	...	564	...	...	...	0		
15	4 p m - 8 p m	...	...	166	477	...	...	...	....	102	4	151	82	19	252
15	8 p m - 12 m	...	...	0	15	...	...	...	....	240	67	755	8	6	15
16	12 m - 4 a m	...	...	1	8	...	...	...	....	149	37	86	0	0	8
16	4 a m - 7 a m	...	...	1	2	...	...	...	....	119	2	64	49	1	10
16	7 a m - 9 a m	...	...	237	63	...	...	...	....	169	5	279	84	3720±	927
16	9 a m - 11 a m	...	...	131	133	...	...	...	....	13	0	58	10	1440±	238
16	11 a m - 1 p m	...	...	314	182	...	...	...	....	3	2	64	7	184	32
16	1 p m - 3 p m	...	...	75	457	...	...	...	....	3	0	5	0	71	8
16	3 p m - 5 p m	...	...	81	192	...	...	...	....	0	0	0	0	13	0
16	5 p m - 7 p m	...	...	5	68	...	...	...	....	0	0	2	208	6	2

TABLE 3.—Summary of the Numbers of Cercaria Elephantis Escaping from Six Specimens of Planorbis Trivolvis

Periods	I	II	III	IV	V	VI
July 6, 6 p m to July 7, 7 p m.....	886	453	15	377	130	397
July 7, 7 p m to July 8, 6 p m..................	2109	945	319	558	314	409
July 8, 6 p m to July 9, 6 p m..................	1427	774	201	584	165	406
July 9, 6 p m to July 10, 6 p m.................	1896	840	439	671	97	268
Total.......................................	6318	3012	974	2190	706	1480

Variations in Different Snails of the Same Species

In the twelve specimens of *Planorbis trivolvis* from which the escape of *Cercaria elephantis* was recorded, there is seen to be a considerable variation in the numbers of cercariae coming out from the different individuals (see Table 1). This point is clearly illustrated from the records of snails I to VI for the four day period from 6 p. m., July 6, to 6 p. m., July 10. The summary of these data (Table 3) shows a great variation in the output of cercariae from these six snails, which during this period were subjected to exactly the same conditions.

That there is a definite relation between the numbers of sporocysts present in a given snail and its output of cercariae was shown by dissections of snails I, V and VI of this series. The comparison of the conditions of the livers of snails I and V, which represent the extremes of cercariae output, was most interesting. Both of these snails were cut open in such a way that the liver was not broken. In both cases the sporocysts were limited to the liver. The liver of snail I was of a uniform pale yellow color, while that of V had a mottled appearance, dark patches being interspersed among the yellow. When the livers of these two snails were carefully examined, it was found that in I all the tissue was packed with a dense mass of tangled sporocysts which had practically replaced all the liver substances. The number of the sporocysts was so great and they were so densely packed and tangled that it was impossible to count them. In snail V it was found that the lighter areas were made up of a large number of sporocysts, but that they were not so densely packed or tangled and that the dark areas between were composed of liver substance which had not been invaded by sporocysts. An estimate of the numbers of sporocysts in these two snails gave I about ten times as many as V, which agrees with the difference in the number of cercariae which escaped. The dissection of snail VI showed that its liver had a somewhat mottled appearance, as in V, but that the dark areas were much smaller, and that the crowding of sporocysts was not nearly as great as I. Therefore VI showed an intermediate condition between I and V.

In a later dissection an attempt was made to count the number of sporocysts in the liver of snail VIII. This count gave approximately 200 sporocysts in this one snail. In the period just before the dissection was made from 3 P. M., July 16, to 6 A. M., July 17, which apparently represented a complete 24-hour cycle for this snail, 337 cercariae were given off. The data given above show that it is possible to correlate the numbers of cercariae given off from any snail with the numbers of sporocysts in its liver.

While the data available are not so extensive the individual variations in the output of the other cercariae studied are just as striking as in the case of *Cercaria elephantis* (see Table 2). For example, in the stylet cercaria series there is a big difference between I and IV. In the case of *C. emarginatae* there is the most striking variation in the whole series in the comparison of II, with a daily output of between 100 and 200, and V, of which is given a record of over 5,000 cercariae given off in one 24-hour period (see Table 2).

Variations in Cercariae Escaping from the Same Snail

From Table I it will be seen that there was a considerable variation on different days in the number of cercariae escaping from the same snail. Of course, variations would be expected in the numbers of

cercariae coming to maturity in the liver of a snail from day to day, and there possibly might have been a small variation due to errors in counting. It is further evident that in the cercaria-producing period of a given infested snail there would be an early period of small cercaria production and a period near the end when the numbers would be diminished. It also seems probable that the great reduction in the cercariae escaping from snails III, IV, IX and XI after August 5 was due to the fact that these snails were badly starved, it being difficult to give them sufficient food under the conditions of the experiment.

It was found also that the temperature of the environment very significantly affected the numbers of cercariae escaping from the snails. On the night of July 5 the temperature dropped to 39.5 F. and the morning of July 6 was cold and rainy. On this day, from 9 A. M., to 6 P. M., only 281 cercariae are recorded as escaping from snail I, as compared with between 1,000 and 2,000 on warmer days. During the other days of the experiment there was a fairly uniform temperature, and no other such marked variations were noted.

To test by experiment the effect of low temperatures on the escape of the cercariae the bottles containing snails IV, XI and XII were kept in water from a well at a temperature ranging from 58 F. to 63 F. from 8:30 P. M., July 19, to 6 P. M., July 20. As shown on the records for July 17 (Table 1), the cercariae were accustomed to escape from these three snails from 6 A. M. to 6 P. M. Therefore, these snails had been kept at low temperatures for the whole night before their cycle usually commenced and during one complete cycle. Examinations were made during this day and it was found that the escape of the cercariae was almost completely inhibited (see records for July 20 on Table 4). From 8 A. M. to 6:45 P. M. on June 20 only 22 cercariae escaped from snail IV; 18 from XI, and 5 from XII, as compared with the normal cycle on July 17, when during approximately the same period 323 cercariae were recorded from IV, 147 from XI and 94 from XII. At 6 P. M. on July 20, the bottles were removed from the cold water and as the temperature at this time was 74 F. they soon warmed up. This increase in temperature was immediately reflected in the escape of cercariae, as is shown by the records from 6:45 P. M. to 9:30 P. M. and from 9:30 P. M. to 6:30 A. M. (see Table 4 records for July 20 and 21), since in all three of these snails there was a considerable number of cercariae found at a time when in their normal cycle practically no cercariae would escape. The cycle of escape of cercariae then resumed its daylight character, as is shown by the records from July 21 and 22. This experiment showed that a temperature only slightly below normal will inhibit the escape of cercariae from their snail hosts. It therefore seems probable that temperature is an important factor in regulating the escape of cercariae from their snail hosts. The investigations just

recorded are merely suggestive and should be carried out much further for various species of cercariae. Judged from these findings, however, it may well be that the infectivity to man of waters in which live snails that harbor the cercariae of the human schistosomes, may be found to be profoundly influenced by temperature.

The Time at Which Cercariae Escape from the Snails

Early in my work on the escape of *Cercaria elephantis* from *Planorbis trivolvis,* I found that the escape of the cercariae did not extend over the whole 24 hours, but that there were periods when they escaped in numbers followed by periods during which none escaped. In other words, the escape came in waves recurring every 24 hours and covering only a part of this period. Perhaps the most surprising finding was that the time of these waves differed in different snails, in some occurring in the daytime and in others at night. Further, it was

TABLE 4.—Number of Cercariae Escaping from Three Snails, Kept at a Temperature Ranging Between 58° and 63° F., from 8:30 P. M., July 19, to 6 P. M., July 20

Day	Hour	IV	XI	XII
July 20	8 a m – 12 n	14	11	2
July 20	12 n – 3 p m	7	2	2
July 20	3 pm – 6:45 p m	1	5	1*
July 20	6:45 p m – 9:30 p m	88	69	59
July 20–21	9:30 p m – 6:30 a m	82	71	15
July 21	6:30 a m – 9 a m	86	41	31
July 21	9 a m – 12 n	121	100	57
July 21	12 n – 3 p m	106	96	61
July 21	3 p m – 6:45 p m	118	115	163
July 21	6:45 p m – 9:30 p m	16	27	16
July 21-22	9:30 p m – 6:45 a m	17	21	7
July 22	6:45 a m – 9:30 a m	74	62	28
July 22	9:30 a m – 12 n	30	110	50
July 22	12 n – 3 p m	49	67	87

* End of period in cold water.

found that the time of these waves suffered but slight change on the different days on which a given snail was studied. For example, in snail I the escape of the cercariae came in the daytime and was pretty largely limited to a period from 6 A. M. to 6 P. M., with the largest numbers escaping from 9 A. M. to 3 P. M. This cycle remained constant during the seven days in which this form was studied. In II the period extended from about 3 P. M. to midnight; in III from about 6 P. M. to 3 A. M.; in IV from about 6 A. M. to 6 P. M.; in V from about 6 P. M. to 3 A. M.; and in VI from about 6 A. M. to 6 P. M. In the other snails of this species studied the same type of cycle of escape of cercariae with its variations in different specimens can be traced (see Table 1). While there are certain slight variations in these waves, their constancy even over a considerable period is very striking. I have no explanation to offer for this phenomenon, which can only have its origin in a periodicity of development of the cercariae themselves.

This same type of periodicity is manifested by *Cercaria emarginatae* in its escape from *Lymnaea emarginata angulata,* as can be seen from Table 2. *C. emarginatae* is also a forked-tailed cercaria, although the presence of a pharynx places it in a different group from *C. elephantis.*

The studies on the echinostome cercaria and the stylet cercaria were made chiefly to compare their cycles with the two forked-tailed species. The cycles of escape of these cercariae from their snail hosts were found to be quite different (see Table 2). In the echinostome species the escape of the cercariae was almost entirely limited to the daytime. In this connection it is interesting to note that echinostome cercariae show a very striking positive reaction to light. The stylet form studied escaped from its snail host during the whole 24 hours, although there was a distinctly smaller number which escaped during the night than during the day (see Table 2).

The results of the experiments outlined above on the escape of cercariae from their snail hosts are in many cases more suggestive than conclusive. They certainly show, however, that further work along this line will be profitable. Since the purpose of the free life of the cercariae is entrance into the definitive host, it is very possible that a study of this same problem for the cercariae of the human trematodes may shed light on the general problem of human infestation and give data of value in control.

Literature Cited

Cort, W. W. 1917.—Homologies of the Excretory System of the Forked-Tailed Cercariae. Jour. Parasit., 4: 49-57.

NEW HUMAN PARASITES

Nicollia aggregata Kritschewsky, 1922.—In four cases of typhus fever, 5 to 14 days after the beginning of the illness, a supposed microorganism interpreted as a protozoan of a previously undescribed species was found in cerebro-spinal fluid obtained by lumbar puncture. Kritschewsky believes this supposed organism is very probably the cause of typhus fever. Two distinct forms were observed. One form usually elongated, sometimes round, measuring about 1.8 μ, possesses a well-defined envelope; the nucleus is usually rod-shaped, sometimes oval, placed perpendicular to the long axis of the cell near its middle. Division stages were observed, the division being perpendicular to the long axis of the cell. The other form measuring about 0.9 μ is entirely without an envelope. [The description and photomicrographs of *N. aggregata* suggest similarities with the bodies found by various observers in the central nervous system in cases of lethargic encephalitis.] (Centralbl. Bakt. Parasit., 1 Abt., Orig., 87 : 526-532, figs. 1-5.)

Embadomonas (*Waskia*) *sinensis* Faust et Wassell, 1921.—Pyriform to elongate oval in shape with two anterior flagella, one directed anteriad and the other posteriad. Cytostome more elongated and nucleus much smaller than in Chilomastix. Size 14 by 4.2 μ. Movement a forward spiral glide with rapid turning, at times somewhat similar to that of Chilomastix, but of less pronounced degree. Reproduction by longitudinal fission; cysts oval-elongate, 6 by 3 μ. In nine cases of diarrhoeic stools of Chinese in Wuchung and of foreigners in Kuling; "probably not as pathogenic as *Giardia intestinalis*." According to the authors, the new flagellate differs from *Embadomonas intestinalis* in shape, size and specific structure. (China Med. Jour., 35 : 543.)

Haemogregarina elliptica Sergent, Sergent and Parrot, 1922.—This new human parasite was found in a Corsican girl, 3 years of age. It has the following characters: Form generally elliptical, homopolar, without reflected tail. Nucleus median and marginal; chromatoid granules disseminated in the cytoplasm. Length of the parasite varies from 1 to 11 μ, breadth about one-third the length. It occurs either in the erythrocytes or in the blood plasma. Some of the extraglobular elements in the peripheral blood show incipient schizogony by transverse division, others are encysted. This parasite is distinguished from two other hemogregarines from man, *Haemogregarina* sp. Krempf, 1917, and *H. inexpectata* Roubaud, 1919. The forms described by Fedorovitch in 1916 in the circulating blood of a case of splenomegaly and considered by Castellani to belong to the genus Toxoplasma seem to Sergent, Sergent and Parrot more nearly related to *H. elliptica* than to the toxoplasms. (Bull. Soc. Path. exot., 15 : 193-197; text fig. 1.)

NOTE

Readers of the JOURNAL will learn with sorrow of the death of Sir Patrick Manson in April and of Professor Alphonse Laveran in May. Both were pioneers in parasitology, for Manson demonstrated the transmission of filarial blood parasites by the mosquito and Laveran found the causal agent of malaria in a blood protozoan. These two truly epoch-making discoveries started a flood of contributions to the knowledge of tropical diseases that are making the conquest of the tropics possible and bringing incalculable benefit to the race.

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The actual dates of issue of Volume VIII of THE JOURNAL were as follows:

No. 1, October 7, 1921 No. 3, May 22, 1922
No. 2, January 13, 1922 No. 4, August 7, 1922